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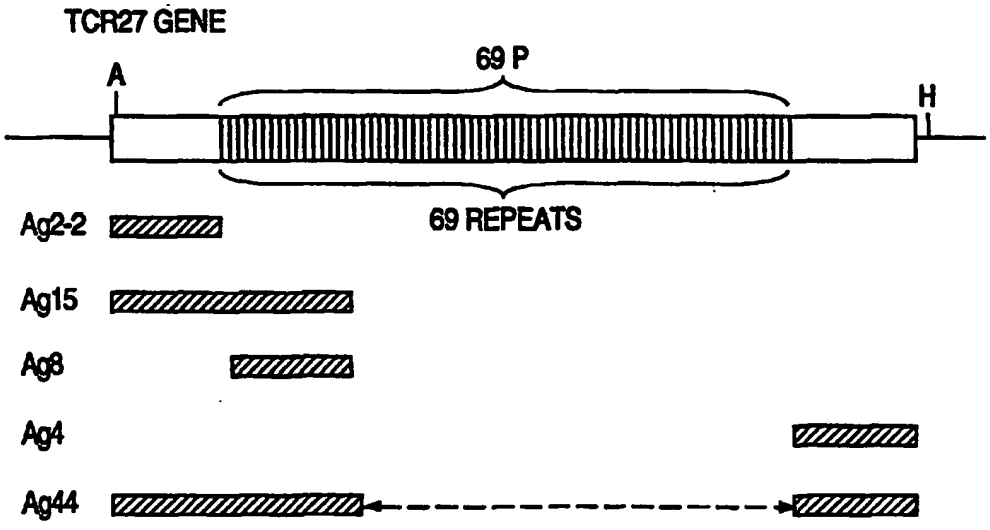

(57) Abstract

Polypeptides are disclosed that are useful for diagnosing American Trypanosomiasis, or Chagas disease, a disease caused by the infectious agent *Trypanosoma cruzi*. The polypeptides have a sequence that corresponds to the amino acid sequence of at least one of the C-terminal and N-terminal nonrepetitive regions of TCR27 protein. The polypeptide additionally may comprise an amino acid sequence of one or more repeats from the central region of TCR27 protein. In a preferred embodiment, the polypeptide corresponds to the N-terminal nonrepetitive region of TCR27 protein and at least one repeat from the central region of TCR27 protein, and does not correspond to the C-terminal nonrepetitive region. The polypeptides may further comprise a linker sequence at either the N-terminus or the C-terminus to facilitate attachment or conjugation to a carrier molecule in a liquid or solid support system for use in a sensitive assay for detecting *T. cruzi* infection.

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POLYPEPTIDES FOR DIAGNOSING INFECTION WITH *TRYPANOSOMA CRUZI*

BACKGROUND OF THE INVENTION

The present invention relates to polypeptides that are useful for diagnosing American trypanosomiasis, or Chagas disease, a disease caused by the infectious agent *Trypanosoma cruzi*. More particularly, the invention relates to recombinant *T. cruzi* polypeptides, synthesized using genetic engineering techniques, and to constructs and processes for producing the recombinant polypeptides, and to an assay for detecting *T. cruzi* infection which employs the polypeptides.

American trypanosomiasis, or Chagas disease, is an illness caused by the protozoan parasite, *T. cruzi (1,2)*. This organism is transmitted by insects called reduviid bugs *(3)*, by blood transfusion *(4)*, and also from mother to fetus *(5)*. Several years after acquiring *T. cruzi* infection, patients may develop the cardiac and gastrointestinal symptoms that are associated with chronic infection, which is life-long, but the majority of infected persons never develop clinical manifestations of Chagas disease and are unaware of being infected. The two drugs available for treating *T. cruzi* infections have low efficacy and often cause serious side effects. In practice, therefore, they have virtually no impact on the control of Chagas disease.

Chagas disease is a major cause of morbidity and death in Latin America, where an estimated 16-18 million people are chronically infected with *T. cruzi (6)*. In recent years tens of thousands of *T. cruzi*-infected people have emigrated to the United States, especially from Central America, where the prevalence of *T. cruzi* infection is high, thus creating the risk of transfusion-associated transmission of the parasite here *(7-9)*. Several such cases have been described *(10-12)*.

Since clinical criteria cannot be depended upon for recognizing *T. cruzi* infection, blood tests are of paramount importance, both in patient care settings and

in blood banks. Chronically infected persons uniformly have anti-*T. cruzi* antibodies. The diagnosis of *T. cruzi* infection is almost always made by detecting these antibodies in patients' blood, since parasitological approaches are laborious and lack sensitivity and, as noted, clinical evaluations lack specificity.

Immunological tests currently used to diagnose *T. cruzi* infection, such as complement fixation and indirect immunofluorescence tests, and enzyme-linked immunosorbent assays (ELISA), often produce inconsistent results and false-positive reactions *(13)*. The occurrence of false-positive reactions can be a problem with specimens from patients with leishmaniasis, schistosomiasis, and other parasitic and infectious diseases, with samples from patients with autoimmune disorders and other illnesses, and with specimens from normal persons.

In large measure these problems with sensitivity and specificity occur because the assays are based on antigens extracted from parasites grown in the laboratory. The complexity and variability of mixtures of native antigens derived from cultured parasites, which persist even after fractionation and purification procedures are used, have been a major barrier to standardization of immunoassays. Because of the limitations of these immunoassays, experts generally agree that blood samples should be positive in three different assays, performed in parallel, before being accepted as positive.

An additional problem related to assays based on material derived from cultured parasites is that producing the antigens creates a serious biohazard for technical personnel, and laboratory-acquired cases of Chagas disease occur with disquieting frequency, both in the United States and abroad *(14,15)*. Furthermore, some of the immunoassays currently available require sophisticated laboratory equipment and levels of technical expertise not generally available in the

countries in which *T. cruzi* infection is endemic.

In response to the need for improved assays for detecting *T. cruzi* infection, considerable work has been invested in the development of new immunoassays. These efforts have accelerated in recent years as new technologies have become available that have the potential for serving as the basis of improved assays. Recombinant DNA technology has led to the molecular cloning of several antigenic *T. cruzi* proteins. Cloned segments of *T. cruzi* genes have been used to produce in bacteria portions of antigenic proteins *(16-22)*. In research settings several of these, singly and in combination, have been used as target antigens in immunoassays. These assays have not been tested in field or blood bank trials, and none is available commercially.

United States patent No. 4,870,006 discloses the use of a recombinant protein in an assay for diagnosing *T. cruzi* infection. A 70-kilodalton heat shock protein constitutes the target antigen in this assay. No information regarding the sensitivity and specificity of the assay is provided in the patent.

In this context, therefore, a need exists for a highly sensitive and specific system for detecting *T. cruzi* infection that is safe, easy, and inexpensive to manufacture and perform.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide a highly sensitive and specific assay for diagnosing infection with *T. cruzi*.

It is a further object of the present invention to provide an assay for diagnosing *T. cruzi* infection that is safe, inexpensive to manufacture and easy to use.

In achieving these and other objects, there has been provided, according to one aspect of the present invention, a polypeptide having a sequence that corresponds to the amino acid sequence of at least one of the C-terminal and N-terminal nonrepetitive regions of the TCR27 protein. The inventive polypeptide

additionally may comprise an amino acid sequence of one or more repeats from the central region of the TCR27 protein. In a preferred embodiment, the polypeptide corresponds to the N-terminal nonrepetitive region of the TCR27 protein and at least one repeat from the central region of the TCR27 protein, and does not correspond to the C-terminal nonrepetitive region. The polypeptides may further comprise a linker sequence at either the N-terminus or the C-terminus to facilitate attachment or conjugation to a carrier molecule in a liquid or solid support system. Isolated polynucleotides that encode the inventive polypeptides according to the present invention are also claimed, as are cells transformed with a recombinant plasmid that expresses a polypeptide according to the invention.

The present invention also provides a method for detecting the presence of antibodies to *T. cruzi* in an individual, comprising the steps of contacting a putative anti-*T. cruzi* antibody-containing sample from an individual with a polypeptide according to the invention that is attached or conjugated to a carrier molecule or attached or conjugated to a solid phase; allowing anti-*T. cruzi* antibodies in said sample to bind to said polypeptide; washing away unbound anti-*T. cruzi* antibodies; and adding a compound that enables detection of the anti-*T. cruzi* antibodies which are specifically bound to the polypeptide. The compound that enables detection of the anti-*T. cruzi* antibodies may be selected from the group consisting of a colorometric agent, a fluorescent agent, a chemiluminescent agent and a radionuclide.

Also provided in accordance with the present invention is a kit for diagnosing the presence of anti-*T. cruzi* antibodies in a sample, comprising a container in which a polypeptide having a sequence that corresponds to the amino acid sequence of at least one of the C-terminal and N-terminal nonrepetitive regions of the TCR27 protein is attached or conjugated to a carrier molecule or

attached or conjugated to a solid phase; and directions for carrying out the method according to the invention. The kit additionally may comprise a container of a compound that binds to anti-*T. cruzi* antibodies and that renders said antibodies detectable.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram of the *T. cruzi* TCR27 gene and the segments of the gene that encode polypeptides according to the present invention.

Figures 2A through 2E show the nucleotide and deduced amino acid sequences (SEQ ID NOS 1-10 res[ectively) of polypeptides according to the present invention.

Figures 3A through 3F are bar graphs of results obtained when recombinant TCR27 polypeptides are used as target antigens in ELISAs to test blood samples (serum or plasma) for anti-*T. cruzi* antibodies.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

It has been discovered that a *T. cruzi* gene designated "TCR27" *(23)* encodes an immunodominant protein containing unique, nonrepetitive regions at both the C-terminus and N-terminus, in addition to a central region comprised of repeats of a 14-amino acid sequence. It has been further discovered that there are two copies of the TCR27 gene that essentially differ only in the number of repeats that comprise the central region. It also has been discovered that the nonrepetitive terminal regions of the TCR27 protein contain epitopes to which individuals infected with *T. cruzi* typically have antibodies. The existence of these epitopes within the

nonrepetitive regions was not suggested previously.

More particularly, the native protein encoded by the TCR27 gene consists of an N-terminal 95-amino acid sequence and a C-terminal 68-amino acid sequence. A central region of repeats encodes 69 repeats of a highly-conserved, 14-amino acid sequence. In accordance with the present invention, a polypeptide that corresponds to at least one of the C-terminal or N-terminal nonrepetitive regions can form the basis for a sensitive assay to diagnose *T. cruzi* infection.

In one preferred embodiment, such a polypeptide corresponds to at least one of the C-terminal or N-terminal nonrepetitive regions in combination with a region of one or more repeats from the central region of the TCR27 protein. In a particularly preferred embodiment, a polypeptide for use in an assay according to the present invention contains the N-terminal nonrepetitive region in combination with one or more repeats from the central region of the TCR27 protein, but does not contain a region corresponding to the C-terminal nonrepetitive region. Polypeptides according to the present invention that include repeat regions in addition to one of the nonrepetitive regions will contain at least one, and preferably at least two, copies of the 14-amino acid repeat.

In addition to the nonrepetitive and repeat regions *per se*, a wide variety of polypeptides which contain the epitopes embodied in these regions can be used in accordance with the present invention. Based on the nucleotide sequences in Figures 2A through 2E (SEQ ID NOS 1, 3, 5. 7 and 9 respectively), polypeptide molecules also can be produced (1) that include sequence variations, relative to the naturally-occurring sequences, (2) that have one or more amino acids truncated from the naturally-occurring sequences and variations thereof, or (3) that contain the naturally-occurring sequences and variations thereof as part of a longer sequence.

In this description, polypeptide molecules in categories (1), (2) and (3) are said to "correspond" to the amino acid sequences of the nonrepetitive or repeat regions of the TCR27 protein. Such polypeptides also are referred to as "variants." The category of variants within the present invention includes, for example, fragments and muteins of the nonrepetitive and repeat regions, as well as larger molecules that consist essentially of one or both of the nonrepetitive sequences, alone or in combination with one or more repeats from the central region.

In this regard, a molecule that "consists essentially of" one or both of the nonrepetitive sequences, alone or in combination with one or more repeats from the central region, is one that reacts immunologically with samples from persons infected with *T. cruzi*, but that does not react with samples from patients with leishmaniasis, schistosomiasis, and other parasitic and infectious diseases, with samples from patients with autoimmune disorders and other illnesses, and with specimens from normal persons.

A "mutein" is a polypeptide that is homologous to the nonrepetitive or repeat region to which it corresponds, and that retains the basic functional attribute -- the ability to react selectively with samples from persons infected with *T. cruzi* -- of the corresponding region. For purposes of this description, "homology" between two sequences connotes a likeness short of identity indicative of a derivation of the first sequence from the second. In particular, a polypeptide is "homologous" to the corresponding nonrepetitive or repeat region if a comparison of amino-acid sequences between the polypeptide and the corresponding region reveals an identity of greater than 70%. Such a sequence comparison can be performed via known algorithms, such as the one described by Lipman and Pearson *(24)*, which are readily implemented by computer. Polypeptides derived from other strains and clones of *T. cruzi* that are homologous to the

sequences shown in Figures 2A through 2E constitute naturally-occurring muteins and are within the scope of the present invention.

A fragment of a nonrepetitive or repeat region is a molecule in which one or more amino acids are truncated from that nonrepetitive or repeat region. Muteins and fragments can be produced, in accordance with the present invention, by known *de novo* synthesis techniques.

Also exemplary of variants within the present invention are molecules that are longer than a nonrepetitive or a repeat region but that contain the region or a mutein thereof within the longer sequence. For example, a variant may include a fusion partner in addition to the nonrepetitive or repeat region. Such a fusion partner may allow easier purification of recombinantly-produced polypeptides. For example, use of a glutathione-S-transferase (26 kilodaltons, GST) fusion partner allows purification of recombinant polypeptides on glutathione agarose beads.

The portion of the sequence of such molecule other than that portion of the sequence corresponding to the region may or may not be homologous to the sequence of the TCR27 protein. If it is homologous with the TCR27 protein, it is not coincident with the sequence of the TCR27 protein.

It will be appreciated that polypeptides shorter than the corresponding nonrepetitive region but that retain the ability to react selectively with samples from persons infected with *T. cruzi* are suitable for use in the present invention. Thus, variants may be of the same length, longer than or shorter than the nonrepetitive or repeat regions, and also include sequences in which there are amino acid substitutions of the parent sequence. These variants must retain the ability to react selectively with samples from persons infected with *T. cruzi*.

Whether a polypeptide based on one of the sequences shown in Figures 2A through 2E (SEQ ID NOS 1-10

respectively) retains the ability to react selectively with samples from persons infected with *T. cruzi* can be determined routinely in accordance with the protocols set forth herein, that is, by reacting it with serologically well-characterized specimens from patients known to be infected with *T. cruzi*, and with similarly serologically well-characterized specimens from patients known to be affected with those conditions that typically cause false positive reactions in assays for antibodies to *T. cruzi*, such as leishmaniasis, schistosomiasis, and other parasitic and infectious diseases, with samples from patients with autoimmune disorders and other illnesses, and with specimens from normal persons.

A schematic diagram of the TCR27 gene is shown in Figure 1. The horizontal rectangle depicts the protein encoding region of the TCR27 gene, which contains a central segment consisting of approximately 69 highly conserved repeats, each 42 nucleotides in length, flanked on both sides by dissimilar, nonrepetitive sequences. Restriction sites are indicated by A (*Ava*II), P (*Pvu*II), and H (*Hinc*III). The positions of the segments of the TCR27 gene that encode polypeptides which are representative of the present invention are indicated by the solid horizontal bars. Thus, polypeptide Ag2-2 is encoded by the nonrepetitive, upstream DNA segment of the TCR27 gene, polypeptide Ag15 by that nonrepetitive segment plus 16 of the 42-nucleotide repeat units, polypeptide Ag8 by a segment consisting of 15 of the 42-nucleotide repeat units, and polypeptide Ag4 by the nonrepetitive, downstream segment of the TCR27 gene. Also, the coding region for polypeptide Ag44 consists of the nonrepetitive, upstream coding region of the TCR27 gene, followed by a segment containing 16 repeats, followed by the nonrepetitive, downstream coding region of the TCR27 gene. The dashed double arrow indicates that the two depicted segments of Ag44 are combined in one continuous coding sequence.

Figure 2A through Figure 2E show the nucleotide and

deduced amino acid sequences (SEQ ID NOS 1-10 respectively) for Ag15, Ag2-2, Ag4, Ag44 and Ag8, respectively. The DNA letter codes are: A, adenine; C, cytosine, G, guanine, and T, thymine. The amino acid codes are: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine. Stop codons are indicated by a single asterisk.

The five TCR27 gene segments that encode recombinant polypeptides according to the invention are inserted into plasmid pGEX *(25)*. The gene encoding GST is positioned upstream from the *Sma*I site into which the TCR27 segments are inserted, and thus the recombinant polypeptides encoded by these plasmids have GST attached to their N-termini. The presence of GST allows purification of the recombinant polypeptides on glutathione agarose beads, as described below, but it will be readily apparent to those of ordinary skill in the art that the GST fusion partner can be cleaved from polypeptides to be used in an assay according to the invention.

Figure 2A shows DNA and deduced amino acid sequences (SEQ ID NOS 1 and 2 respectively) of Ag15, which is a GST-TCR27 polypeptide-pGEX-2T polylinker fusion protein. GST is encoded by nucleotides 1 through 681, which are derived from pGEX-2T. The segment of the *T. cruzi* TCR27 protein that constitutes part of Ag15 is encoded by nucleotides 682 through 1671. The seven-amino acid sequence that constitutes the C-terminus of Ag15 is encoded by nucleotides 1672 through 1695, which is the pGEX-2T polylinker remnant that lies downstream from the *Sma*I site.

Figure 2B shows DNA and deduced amino acid sequences (SEQ ID NOS 3 and 4 respectively) of Ag2-2, which is a GST-TCR27 polypeptide-pGEX-2T polylinker fusion protein. GST is encoded by nucleotides 1 through 681, which are

derived from pGEX-2T. The segment of the *T. cruzi* TCR27 protein that constitutes part of Ag2-2 is encoded by nucleotides 682 through 1041. The seven-amino acid sequence that constitutes the C-terminus of Ag2-2 is encoded by nucleotides 1042 through 1065 which is the pGEX-2T polylinker remnant that lies downstream from the *Sma*I site.

Figure 2C shows DNA and deduced amino acid sequences (SEQ ID NOS 5 and 6 respectively) of Ag4, which is a GST-TCR27 polypeptide fusion protein. GST is encoded by nucleotides 1 through 663, which are derived from pGEX-1. The segment of the *T. cruzi* TCR27 protein that constitutes part of Ag4 is encoded by nucleotides 664 through 924.

Figure 2D shows DNA and deduced amino acid sequences (SEQ ID NOS 7 and 8 respectively) of Ag44, which is a GST-TCR27 polypeptide fusion protein. GST is encoded by nucleotides 1 through 681, which are derived from pGEX-2T. The segment of the *T. cruzi* TCR27 protein that constitutes part of Ag44 is encoded by nucleotides 682 through 1932.

Figure 2E shows DNA and deduced amino acid sequences (SEQ ID NOS 9 and 10 respectively) of Ag8, which is a fusion protein consisting of the following polypeptides: (1) GST is encoded by nucleotides 1 through 678, which are derived from pGEX-3X; (2) a six-amino acid sequence is encoded by nucleotides 679 through 696, which are derived from the region of the polylinker region of pBluescript *(26)* that lies between the *Bam*HI and *Eco*RI sites; (3) the segment of the *T. cruzi* TCR27 protein that constitutes part of Ag8 is encoded by nucleotides 697 through 1374; (4) a seven-amino acid sequence is encoded by nucleotides 1375 through 1395, which are derived from the region of the polylinker region of pBluescript that lies between the *Eco*RV and *Hinc*II sites ; and (5) a seven-amino acid sequence that constitutes the C-terminus of Ag8 is encoded by nucleotides 1396 through 1419 which is the pGEX-3X polylinker remnant that lies downstream

from the *Hinc*II site.

The presence of GST in these five fusion polypeptides allows purification of the recombinant polypeptides on glutathione agarose beads, as described below, but it will be readily apparent to those of ordinary skill in the art that the GST fusion partner can be cleaved from polypeptides to be used in an assay according to the invention.

Polypeptides useful in an assay according to the invention can be synthetic peptides made by chemical synthesis techniques, but preferably are produced by recombinant techniques. DNA encoding the polypeptides preferably is obtained by cloning and recombination of DNA segments of the TCR27 gene. These DNA segments are utilized to produce recombinant polypeptides in bacteria. The N-termini or the C-termini of these polypeptides can be modified, respectively, to include a linker sequence that facilitates attachment or conjugation of the portions of the polypeptides that constitute the reactive epitopes to carrier molecules in solution or to solid support systems. In addition, the DNA sequences that encode the recombinant polypeptides may be modified such that the amino acid sequences described herein are not altered, or they may be altered such that the polypeptides are shortened or lengthened, or have amino acid substitutions that are preferably conservative.

The present invention further relates to methods for diagnosing *T. cruzi* infection by detecting antibodies that bind specifically to epitopes contained in the inventive polypeptides. The method consists of bringing into contact a sample of whole blood, or an antibody-containing component of blood, with a polypeptide, according to the invention, that is attached or conjugated to a carrier molecule or solid phase. After a period of contact between the sample and the polypeptide, during which antibodies in the sample are bound to the polypeptide, unbound antibodies are washed away. The bound antibodies are then visualized or

otherwise detected by adding a compound or compounds that detect the antibodies which are specifically bound to the polypeptides. Exemplary of compounds that enable detection of the anti-*T. cruzi* antibodies are colorometric agents, fluorescent agents, chemiluminescent agents and radionuclides.

A significant feature of the present invention is that it enables the use of a well-defined *T. cruzi* antigen, to which a large number of infected individuals produce antibodies, in a method of diagnosing *T. cruzi* infection. In accordance with the present invention, preparations formulated from polypeptides which are produced recombinantly or by chemical synthesis, respectively, are "substantially pure." That is, they do not contain other proteins or polypeptides of *T. cruzi* origin, in contrast to antigenic preparations derived from cultured parasites. Such crude preparations are complex and variable in constituency, and typically contain a variety of *T. cruzi* antigens even after fractionation and purification procedures are used. Some of these other antigens are cross-reactive with other antibodies produced in response to other parasitic and infectious diseases, and to some noninfectious diseases as well, giving rise to false positives. This has been a major barrier to standardization of immunoassays for diagnosis of *T. cruzi*.

A high percentage of blood specimens from *T. cruzi*-infected persons from six different Latin American countries had easily demonstrable specific antibodies to polypeptides according to the invention, whereas specimens from normal persons did not. Equally important, specimens from patients with diseases that are often associated with false-positive reactions, such as leishmaniasis, schistosomiasis, and other parasitic and infectious diseases, as well as autoimmune disorders, did not produce false positives in assays with polypeptides according to the present invention. Thus, the present polypeptides are useful for diagnosing infection with

T. cruzi.

Results of assays with various polypeptides are shown in Figures 3A through 3F. Two panels of specimens were used. The first panel consisted of twelve serologically well-characterized specimens from *T. cruzi*-infected patients from six Latin American countries, and twelve control specimens from healthy persons, half from Latin America and half from the United States. The second panel of specimens consisted of twelve serologically well-characterized specimens from *T. cruzi*-infected patients from five Latin American countries, and 44 control specimens from patients with the following conditions (# of patients):

visceral leishmaniasis (8)
cutaneous leishmaniasis (8)
autoimmune disease (6)
schistosomiasis (4)
toxoplasmosis (2)
pneumocystosis (2)
syphilis (1)

and healthy persons (13).

The *T. cruzi*-infected patients in the two panels were not selected because of high or low antibody titers, as determined in conventional immunoassays, and the two groups of twelve *T. cruzi*-infected patients did not overlap.

Figure 3A presents results obtained when Ag15 was reacted with specimens in Panel 2 in an ELISA. The vertical bars indicate mean absorbance values for the *T. cruzi*-infected and uninfected groups. Standard deviations are indicated by the lines projecting from the bars. The ratio of the mean absorbance value of the *T. cruzi*-infected patients to that of the controls was 4:1, suggesting that Ag15 can serve as the basis for sensitive and specific assays for detecting *T. cruzi* infection.

Results obtained when Ag2-2 was reacted with specimens in Panel 1 in an ELISA are shown in Figure 3B.

The ratio of the mean absorbance value of the *T. cruzi*-infected patients to that of the controls was 1.5:1. While this was considerably less than the ratio of absorbance values obtained with Ag15, the results do indicate clearly that many *T. cruzi*-infected patients have antibodies that bind specifically to epitopes present on the nonrepetitive, upstream portion of the TCR27 protein and that Ag2-2 can be used in an assay for detecting *T. cruzi* infection.

Figure 3C shows results obtained when Ag4 was reacted with specimens in Panel 1 in an ELISA. The ratio of the mean absorbance value of the *T. cruzi*-infected patients to that of the controls was 1.7:1. This ratio of absorbance values again was considerably less than the ratio obtained with Ag15, but as was the case with Ag2-2 the results indicate clearly that many *T. cruzi*-infected patients have antibodies that bind specifically to epitopes present on the nonrepetitive, downstream portion of the TCR27 protein and that an assay for detecting *T. cruzi* infection can be based on Ag4.

Results obtained when Ag44 was reacted with specimens in Panel 2 in an ELISA are presented in Figure 3D. The ratio of the mean absorbance value of the *T. cruzi*-infected patients to that of the uninfected persons was 2:1, suggesting that Ag44 can serve as the basis for sensitive and specific assays for detecting *T. cruzi* infection.

Figure 3E displays results obtained when Ag8 was reacted with specimens in Panel 2 in an ELISA. The ratio of the mean absorbance value of the *T. cruzi*-infected patients to that of the controls was 1.5:1. This is less than the ratios obtained with Ag15 and Ag44, thus suggesting that assays based on the latter antigens will be more discriminative than assays based on Ag8.

Results obtained when GST alone was reacted with specimens in Panel 2 in an ELISA are displayed in Figure 3F. The ratio of the mean absorbance value of the *T. cruzi*-infected patients to that of the controls is

1:1, indicating unambiguously that the ability of the assays based on the recombinant TCR27 proteins to discriminate between specimens from *T. cruzi*-infected patients and those of controls is based on antibody binding to the *T. cruzi* portions of the fusion proteins, rather than on reactivity with GST.

The present invention can be understood further with reference the following, non-limiting examples.

Example 1. Propagation and Isolation of Parasites

Epimastigotes of the Sylvio X-10/4 clone of *T. cruzi (27)* were maintained in logarithmic growth phase at 26°C in supplemented liver digest neutralized medium and harvested as described earlier *(28)*. Mixtures of epimastigotes and culture-derived metacyclic trypomastigotes (CMT) (~1:1) were produced in supplemented Grace's insect medium, and purified CMT (>90%) were obtained by passing the mixture through a DE52 column.

Example 2. Construction of cDNA Expression Library

RNA was isolated from purified Sylvio X-10/4 CMT as described *(29)* and cDNAs were synthesized from total RNA, without prior isolation of poly(A)$^+$ RNA, with Moloney murine leukemia virus reverse transcriptase in the BRL Synthesis System (Bethesda Research Laboratories, Gaithersburg, MD). After treatment of the cDNAs with *Eco*RI methylase, *Eco*RI linkers were attached and the cDNAs were ligated into bacteriophage ZAP (Stratagene, San Diego, CA). After packaging of the recombinant phage with GigaPack Gold (Stratagene), a library of 6.4 x 10^6 independent clones was obtained, and 5 x 10^6 clones were amplified in *E. coli* Y1090.

Example 3. Immunoscreening the cDNA Library and Isolation of a TCR27 cDNA

Serum from a Bolivian patient with clinically apparent Chagas disease, whose infection with *T. cruzi* had been established parasitologically and by conventional serologic assays *(30)*, was used for immunoscreening. The amplified cDNA library was immunoscreened as described previously *(31)* using

horseradish peroxidase-conjugated goat anti-immunoglobulin G as secondary antibody. Approximately 30 strongly reactive phage were identified, and recombinant pBluescript plasmids were recovered from purified reactive ZAP clones by coinfecting *E. coli* XL1-Blue with the recombinant phage and R408 helper phage *(26)*. Nucleotide sequences of cloned cDNAs were determined using the Sequenase kit (U.S. Biochemicals, Cleveland, OH).

One of the cDNAs isolated by this approach, designated "TCR27," is 1,660 nucleotides in length and has a 1,230 nucleotide single open reading frame as well as a poly A tail. The upstream segment of this cDNA encodes 25 highly conserved 14-amino acid repeats, and the portion of the coding region downstream from this repetitive region encodes a dissimilar and nonrepetitive 68-amino acid sequence *(17)*.

Example 4. Construction of the Genomic Library and Isolation of a Full-Length TCR27 Gene

Genomic DNA was isolated from 6 x 10^9 Sylvio X-10/4 epimastigotes as described *(32)*. A genomic library was constructed in bacteriophage FIX using the procedures suggested by the supplier of the vector (Stratagene). Approximately 100,000 phage plaques were screened by hybridizing radiolabeled TCR27 cDNA to phage DNA bound to nitrocellulose filters using standard procedures *(33)*. Six recombinant phage-bearing inserts containing at least a segment of a TCR27 gene were identified, and one, which was approximately 9.5 kilobases in length, was characterized in detail after cloning into plasmid pBluescript.

DNA of the pBluescript clone bearing the 9.5 kilobase TCR27 fragment was prepared as described *(33)* and analyzed by digestion with various restriction endonucleases, electrophoresis in 1% agarose gels, and visualization under UV illumination. Information obtained through restriction mapping and DNA sequencing, performed using the Sequenase kit (U.S. Biochemicals) and

on an automated DNA sequencer (ABI, Foster City, CA) was used to construct the schematic diagram of the TCR27 gene shown in Figure 1. The salient features of the TCR27 gene include a ~2.9 kilobase central region that encodes 69 of the highly conserved 14-amino acid repeats. This central region is flanked upstream and downstream by dissimilar and nonrepetitive regions that encode 95- and 68-amino acid sequences respectively.

Example 5. Construction of Recombinant Plasmids Containing Segments of the TCR27 Gene

Plasmid encoding Ag15.

Recombinant pBluescript DNA bearing the TCR27 gene was digested with *Ava*II and *Hinc*II and the resulting 3.8 kilobase fragment, after isolation by electrophoresis and filling in the *Ava*II end, was cloned into the *Sma*I site of pGEX-2T (Pharmacia Biotech, Piscataway, NJ) *(25)* using standard procedures *(33)*. After production of DNA of the latter recombinant plasmid, designated pTCR27-7, a *Bam*HI-*Eco*RI fragment was isolated and was subjected to partial digestion with *Pvu*II, which cuts in the 42-nucleotide TCR27 repeat sequence. The resulting mixture of DNA fragments containing variable numbers of repeats was then cloned into pGEX-2T which had been digested previously with *Sma*I and *Bam*HI. After cloning of the resulting recombinant plasmids, the sizes of their inserts were determined by *Bam*HI-*Eco*RI digestion and electrophoresis. A plasmid containing a ~850 nucleotide insert, designated pGEX-2T-Ag15, was selected for further evaluation. The presence at the upstream end of this insert of the 5' nonrepetitive segment of the TCR27 coding region and the 42-nucleotide repeats at its 3' terminus was confirmed by DNA sequencing, as was the in-frame positioning of the region that encodes the recombinant protein. When Ag15 was produced in *E. coli* as described below, a protein of the expected size was present in a Coomassie blue-stained gel, and this protein reacted with an anti-TCR27 repeat serum in a Western blot. This latter serum was produced by immunizing a rabbit with a synthetic peptide

consisting of two 14-amino acid TCR27 repeats.

Plasmid encoding Ag44.

Beginning with pTCR27-7 DNA (see Ag15 above) a *Bam*HI-*Eco*RI fragment was isolated and subjected to partial digestion with *Pvu*II and fragments ~0.5-0.75 kilobases were isolated from the resulting mixture. This mixture of fragments was then treated with ligase to generate *Bam*HI-*Eco*RI fragments similar to the native TCR27 coding region, but with far fewer repeats in their central regions. The resulting fragments were then cloned into pGEX-2T previously digested with *Bam*HI and *Eco*RI. The sizes of the inserts in the resulting recombinant plasmids were determined by *Bam*HI and *Eco*RI digestion and electrophoresis, and one containing a ~1.1 kilobase insert, designated pGEX-2T-Ag44, was selected for further evaluation. The presence at the upstream end of this insert of the 5' nonrepetitive segment of the TCR27 coding region and the 3' nonrepetitive segment at its 3' terminus, as well as the presence of an intervening region of repeats, was confirmed by DNA sequencing. In addition, the in-frame positioning of the 5' end of the coding region of the construct was confirmed by this approach. When Ag44 was produced in *E. coli* as described below, a protein of the expected size was present in a Coomassie blue-stained gel, and this protein reacted with the anti-TCR27 repeat serum in a Western blot.

Plasmid encoding Ag2-2.

pGEX-2T-Ag44 DNA was digested to completion with *Bam*HI and *Pvu*II, and fragments ~350 nucleotides in length were cloned into pGEX-2T previously digested with *Bam*HI and *Sma*I. The presence in one of the resulting plasmids of the 5' nonrepetitive coding region of the TCR27 gene was confirmed by DNA sequencing, as was a lack of repeats and the in-frame positioning of the insert. As with the other recombinant antigens, an appropriately sized protein was produced in *E. coli*.

Plasmid encoding Ag4.

pGEX-2T-Ag44 DNA was digested to completion with

*Pvu*II and *Eco*RI, and fragments ~350 nucleotides in length were cloned into pGEX-1 previously digested with *Sma*I and *Eco*RI. The presence in one of the resulting plasmids of the 3' nonrepetitive coding region of the TCR27 gene was confirmed by DNA sequencing, as was a lack of repeats and the in-frame positioning of the insert. As with the other recombinant antigens, an appropriately sized protein was produced in *E. coli*.

Plasmid encoding Ag8.

An *Eco*RI-*Hinc*II fragment of the TCR27 cDNA was cloned into pBluescript SK that had been previously digested with these two endonucleases. The resulting recombinant plasmid was linearized with *Hinc*II and then digested with Bal 31 with the purpose removing the 3' nonrepetitive region while leaving a region of repeats. A fragment obtained by this approach was shown to have a segment containing ~700 nucleotides of repetitive sequence and was cloned into pBluescript. The presence of repeats at both ends of this insert was confirmed by DNA sequencing. The insert, as a *Bam*HI-*Hinc*II fragment, was then excised from pBluescript and cloned into the *Bam*HI-*Sma*I site of pGEX-3X. When Ag8 was produced in *E. coli* a protein of the expected size was seen in a Coomassie blue stained gel, and this protein reacted with antibodies in the anti-TCR27 repeat serum.

Example 6. Expression in *E. coli* and Purification of Recombinant Polypeptides

For the production of recombinant polypeptides, *E. coli* DH5∫ transformed with pGEX bearing a TCR27 coding segment, was grown overnight at 37°C in liquid LB medium containing 100 µg/ml ampicillin. One-tenth volume of this culture was then inoculated into approximately 80 ml fresh LB/amp medium, and after incubation for 1 hour, isopropyl-ß-D-thiogalactopyranoside was added to a concentration of 0.1 mM and the culture was further incubated for 3-7 hours at 37°C. The culture was then centrifuged at 3,000 x g for 15 minutes at 4°C, and after aspiration of the supernatant the pellet was suspended to

2.5 ml in phosphate buffered saline (PBS) containing 1% Triton X-100 and 1.6 mM phenylmethylsulfonyl fluoride to inhibit proteolysis. The cell suspension was sonicated until it became bubbly and then centrifuged at 10,000 x g for 10 minutes.

Partial purification of the recombinant polypeptides was accomplished by mixing the above supernatant with 200µl of 50% glutathione-agarose beads (Sigma, St. Louis, MO) suspended in PBS and incubating at room temperature for 1 hour with gentle shaking. The beads were then washed 2 times with 0.5% Triton X-100 and 1.6 mM phenylmethylsulfonyl fluoride in PBS, followed by a single wash with PBS. To remove the recombinant protein from the beads, 200 µl of 10 mM glutathione in 50 mM Tris-HCl, pH 8 was added and incubated for 10 minutes at room temperature with gentle shaking, and the beads are pelleted in a microcentrifuge. This procedure was repeated once and the supernatants obtained were combined, after which the protein concentration was determined using a protein assay kit (Bio-Rad, Richmond, CA).

Example 7. ELISA for Detecting *T. cruzi* Infection

To test blood samples for antibodies that bind specifically to the recombinant *T. cruzi* antigens, the following procedure was employed. After purification on glutathione agarose, the recombinant antigen was diluted in PBS to a concentration of 5 ug/ml (500 ng/100 µl). One hundred microliters of the diluted antigen solution was added to each well of a 96-well Immulon 1 plate (Dynatech Laboratories, Chantilly, VA), and the plate was then incubated for 1 hour at room temperature, or overnight at 4°C, and washed 3 times with 0.05% Tween 20 in PBS. Blocking to reduce nonspecific binding of antibodies was accomplished by adding to each well 200 µl of a 1% solution of bovine serum albumin in PBS/Tween 20 and incubation for 1 hour. After aspiration of the blocking solution, 100 µl of the primary antibody solution (anticoagulated whole blood, plasma, or serum),

diluted in the range of 1/16 to 1/2048 in blocking solution, was added and incubated for 1 hour at room temperature or overnight at 4°C. The wells were then washed 3 times, and 100 µl of goat anti-human IgG antibody conjugated to horseradish peroxidase (Organon Teknika, Durham, NC), diluted 1/500 or 1/1000 in PBS/Tween 20, 100 µl of o-phenylenediamine dihydrochloride (OPD, Sigma) solution was added to each well and incubated for 5-15 minutes. The OPD solution was prepared by dissolving a 5 mg OPD tablet in 50 ml 1% methanol in H_2O and adding 50 µl 30% H_2O_2 immediately before use. The reaction was stopped by adding 25 l of 4M H_2SO_4. Absorbances were read at 490 nm in a microplate reader (Bio-Rad).

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(ii) TITLE OF INVENTION: POLYPEPTIDES FOR DIAGNOSING INFECTION WITH TRYPANOSOMA CRUZI

(iii) NUMBER OF SEQUENCES: 10

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(v) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: NOT YET ASSIGNED
(B) FILING DATE: CONCURRENTLY HEREWITH

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER US 08/216,894
(B) FILING DATE: 24-MAR-1994

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1695 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1692

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 564 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Glu Lys Leu Asp Ala Ile Asn Arg Ala Thr Lys Leu Glu Glu Glu Arg
            260                 265                 270

Asn Gln Ala Tyr Lys Ala Ala His Lys Ala Glu Glu Glu Lys Ala Lys
        275                 280                 285

Thr Phe Gln Arg Leu Ile Thr Phe Glu Ser Glu Asn Ile Asn Leu Lys
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Ala Lys Ala Val Glu Thr Glu Lys Gln Arg Ala Ala Glu Ala Thr Lys
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Val Ala Glu Ala Glu Lys Arg Lys Ala Ala Glu Ala Ala Lys Ala Val
        355                 360                 365

Glu Thr Glu Lys Gln Arg Ala Ala Glu Ala Thr Lys Val Ala Glu Ala
    370                 375                 380

Glu Lys Gln Lys Ala Ala Glu Ala Ala Lys Ala Val Glu Thr Glu Lys
385                 390                 395                 400

Gln Arg Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Arg
                405                 410                 415

Ala Ala Glu Ala Met Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala
            420                 425                 430

Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala
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Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys
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Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala
465                 470                 475                 480

Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala Glu Ala
                485                 490                 495

Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys
            500                 505                 510

Gln Lys Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys
        515                 520                 525

Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala
    530                 535                 540

Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Gly Glu Phe
545                 550                 555                 560

Ile Val Thr Asp
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1065 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1062

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATG TCC CCT ATA CTA GGT TAT TGG AAA ATT AAG GGC CTT GTG CAA CCC        48
Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro
 1               5                  10                  15

ACT CGA CTT CTT TTG GAA TAT CTT GAA GAA AAA TAT GAA GAG CAT TTG        96
Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
            20                  25                  30

TAT GAG CGC GAT GAA GGT GAT AAA TGG CGA AAC AAA AAG TTT GAA TTG       144
Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
        35                  40                  45

GGT TTG GAG TTT CCC AAT CTT CCT TAT TAT ATT GAT GGT GAT GTT AAA       192
Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
    50                  55                  60

TTA ACA CAG TCT ATG GCC ATC ATA CGT TAT ATA GCT GAC AAG CAC AAC       240
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65                  70                  75                  80

ATG TTG GGT GGT TGT CCA AAA GAG CGT GCA GAG ATT TCA ATG CTT GAA       288
Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
                85                  90                  95

GGA GCG GTT TTG GAT ATT AGA TAC GGT GTT TCG AGA ATT GCA TAT AGT       336
Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
            100                 105                 110

AAA GAC TTT GAA ACT CTC AAA GTT GAT TTT CTT AGC AAG CTA CCT GAA       384
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
        115                 120                 125

ATG CTG AAA ATG TTC GAA GAT CGT TTA TGT CAT AAA ACA TAT TTA AAT       432
Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
    130                 135                 140

GGT GAT CAT GTA ACC CAT CCT GAC TTC ATG TTG TAT GAC GCT CTT GAT       480
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145                 150                 155                 160

GTT GTT TTA TAC ATG GAC CCA ATG TGC CTG GAT GCG TTC CCA AAA TTA       528
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
                165                 170                 175

GTT TGT TTT AAA AAA CGT ATT GAA GCT ATC CCA CAA ATT GAT AAG TAC       576
Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
            180                 185                 190

TTG AAA TCC AGC AAG TAT ATA GCA TGG CCT TTG CAG GGC TGG CAA GCC       624
Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
        195                 200                 205

ACG TTT GGT GGT GGC GAC CAT CCT CCA AAA TCG GAT CTG GTT CCG CGT       672
Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
    210                 215                 220

GGA TCC CCG TCC CAG CTC CAA CAG GCA GAA AAT AAT ATC ACT AAT TCC       720
Gly Ser Pro Ser Gln Leu Gln Gln Ala Glu Asn Asn Ile Thr Asn Ser
225                 230                 235                 240

AAA AAA GAA ATG ACA AAG CTA CGA GAA AAA GTG AAA AAG GCC GAG AAA       768
Lys Lys Glu Met Thr Lys Leu Arg Glu Lys Val Lys Lys Ala Glu Lys
                245                 250                 255
```

```
GAA AAA TTG GAC GCC ATT AAC CGG GCA ACC AAG CTG GAA GAG GAA CGA        816
Glu Lys Leu Asp Ala Ile Asn Arg Ala Thr Lys Leu Glu Glu Glu Arg
            260                 265                 270

AAC CAA GCG TAC AAA GCA GCA CAC AAG GCA GAG GAG GAA AAG GCT AAA        864
Asn Gln Ala Tyr Lys Ala Ala His Lys Ala Glu Glu Glu Lys Ala Lys
        275                 280                 285

ACA TTT CAA CGC CTT ATA ACA TTT GAG TCG GAA AAT ATT AAC TTA AAG        912
Thr Phe Gln Arg Leu Ile Thr Phe Glu Ser Glu Asn Ile Asn Leu Lys
    290                 295                 300

AAA AGG CCA AAT GAC GCA GTT TCA AAT CGG GAT AAG AAA AAA AAT TCT        960
Lys Arg Pro Asn Asp Ala Val Ser Asn Arg Asp Lys Lys Lys Asn Ser
305                 310                 315                 320

GAA ACC GCA AAA ACT GAC GAA GTA GAG AAA CAG AGG GCG GCT GAG GCT       1008
Glu Thr Ala Lys Thr Asp Glu Val Glu Lys Gln Arg Ala Ala Glu Ala
                325                 330                 335

GCC AAG GCC GTG GAG ACG GAG AAG CAG AGG GCA GGG GAA TTC ATC GTG       1056
Ala Lys Ala Val Glu Thr Glu Lys Gln Arg Ala Gly Glu Phe Ile Val
            340                 345                 350

ACT GAC TGA                                                           1065
Thr Asp
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 354 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro
 1               5                  10                  15

Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
            20                  25                  30

Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
        35                  40                  45

Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
    50                  55                  60

Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65                  70                  75                  80

Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
                85                  90                  95

Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
            100                 105                 110

Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
        115                 120                 125

Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
    130                 135                 140
```

```
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145                 150                 155                 160

Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
                165                 170                 175

Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
            180                 185                 190

Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
        195                 200                 205

Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
    210                 215                 220

Gly Ser Pro Ser Gln Leu Gln Gln Ala Glu Asn Asn Ile Thr Asn Ser
225                 230                 235                 240

Lys Lys Glu Met Thr Lys Leu Arg Glu Lys Val Lys Lys Ala Glu Lys
                245                 250                 255

Glu Lys Leu Asp Ala Ile Asn Arg Ala Thr Lys Leu Glu Glu Glu Arg
            260                 265                 270

Asn Gln Ala Tyr Lys Ala Ala His Lys Ala Glu Glu Glu Lys Ala Lys
        275                 280                 285

Thr Phe Gln Arg Leu Ile Thr Phe Glu Ser Glu Asn Ile Asn Leu Lys
    290                 295                 300

Lys Arg Pro Asn Asp Ala Val Ser Asn Arg Asp Lys Lys Lys Asn Ser
305                 310                 315                 320

Glu Thr Ala Lys Thr Asp Glu Val Glu Lys Gln Arg Ala Ala Glu Ala
                325                 330                 335

Ala Lys Ala Val Glu Thr Glu Lys Gln Arg Ala Gly Glu Phe Ile Val
            340                 345                 350

Thr Asp
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 924 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..921

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
ATG TCC CCT ATA CTA GGT TAT TGG AAA ATT AAG GGC CTT GTG CAA CCC      48
Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro
  1               5                  10                  15

ACT CGA CTT CTT TTG GAA TAT CTT GAA GAA AAA TAT GAA GAG CAT TTG      96
Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
            20                  25                  30
```

```
TAT GAG CGC GAT GAA GGT GAT AAA TGG CGA AAC AAA AAG TTT GAA TTG          144
Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
          35                  40                  45

GGT TTG GAG TTT CCC AAT CTT CCT TAT TAT ATT GAT GGT GAT GTT AAA          192
Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
      50                  55                  60

TTA ACA CAG TCT ATG GCC ATC ATA CGT TAT ATA GCT GAC AAG CAC AAC          240
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
 65                  70                  75                  80

ATG TTG GGT GGT TGT CCA AAA GAG CGT GCA GAG ATT TCA ATG CTT GAA          288
Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
                85                  90                  95

GGA GCG GTT TTG GAT ATT AGA TAC GGT GTT TCG AGA ATT GCA TAT AGT          336
Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
           100                 105                 110

AAA GAC TTT GAA ACT CTC AAA GTT GAT TTT CTT AGC AAG CTA CCT GAA          384
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
       115                 120                 125

ATG CTG AAA ATG TTC GAA GAT CGT TTA TGT CAT AAA ACA TAT TTA AAT          432
Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
   130                 135                 140

GGT GAT CAT GTA ACC CAT CCT GAC TTC ATG TTG TAT GAC GCT CTT GAT          480
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145                 150                 155                 160

GTT GTT TTA TAC ATG GAC CCA ATG TGC CTG GAT GCG TTC CCA AAA TTA          528
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
               165                 170                 175

GTT TGT TTT AAA AAA CGT ATT GAA GCT ATC CCA CAA ATT GAT AAG TAC          576
Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
           180                 185                 190

TTG AAA TCC AGC AAG TAT ATA GCA TGG CCT TTG CAG GGC TGG CAA GCC          624
Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
       195                 200                 205

ACG TTT GGT GGT GGC GAC CAT CCT CCA AAA TCG GAT CCC CCT GAA GCT          672
Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Pro Pro Glu Ala
   210                 215                 220

GCC AAG GCT ATG GAG TCG CAG AAG CAG AGA TTC TTA GAA CGT TTT GCG          720
Ala Lys Ala Met Glu Ser Gln Lys Gln Arg Phe Leu Glu Arg Phe Ala
225                 230                 235                 240

GTT CTT GAG GAG GAG AAA AAG GCA GCC TTA AGA GCG GCG GAG ATG GAG          768
Val Leu Glu Glu Glu Lys Lys Ala Ala Leu Arg Ala Ala Glu Met Glu
               245                 250                 255

AGG AGG AAA ATA ACA AAC ATA ATG AAG AAT AAA GGT GTA CGC AGT TCG          816
Arg Arg Lys Ile Thr Asn Ile Met Lys Asn Lys Gly Val Arg Ser Ser
           260                 265                 270

GAT TCG GTG CCG CTT GTG GAG GGG AAT CGC TCT GTT ACT GAG AGT TCT          864
Asp Ser Val Pro Leu Val Glu Gly Asn Arg Ser Val Thr Glu Ser Ser
       275                 280                 285

TGT AGA AAT CGG TTT CGT TTT TGT AGA AAT CGG TTT CGT TTT TCA TGT          912
Cys Arg Asn Arg Phe Arg Phe Cys Arg Asn Arg Phe Arg Phe Ser Cys
   290                 295                 300
```

TCT GTA ATG TGA 924
Ser Val Met
305

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 307 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro
1 5 10 15

Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
20 25 30

Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
35 40 45

Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
50 55 60

Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65 70 75 80

Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
85 90 95

Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
100 105 110

Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
115 120 125

Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
130 135 140

Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145 150 155 160

Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
165 170 175

Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
180 185 190

Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
195 200 205

Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Pro Pro Glu Ala
210 215 220

Ala Lys Ala Met Glu Ser Gln Lys Gln Arg Phe Leu Glu Arg Phe Ala
225 230 235 240

Val Leu Glu Glu Glu Lys Lys Ala Ala Leu Arg Ala Ala Glu Met Glu
245 250 255

Arg Arg Lys Ile Thr Asn Ile Met Lys Asn Lys Gly Val Arg Ser Ser
260 265 270

```
Asp Ser Val Pro Leu Val Glu Gly Asn Arg Ser Val Thr Glu Ser Ser
      275                 280                 285

Cys Arg Asn Arg Phe Arg Phe Cys Arg Asn Arg Phe Arg Phe Ser Cys
    290                 295                 300

Ser Val Met
305
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1932 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1929

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
ATG TCC CCT ATA CTA GGT TAT TGG AAA ATT AAG GGC CTT GTG CAA CCC        48
Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro
  1               5                  10                  15

ACT CGA CTT CTT TTG GAA TAT CTT GAA GAA AAA TAT GAA GAG CAT TTG        96
Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
            20                  25                  30

TAT GAG CGC GAT GAA GGT GAT AAA TGG CGA AAC AAA AAG TTT GAA TTG       144
Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
        35                  40                  45

GGT TTG GAG TTT CCC AAT CTT CCT TAT TAT ATT GAT GGT GAT GTT AAA       192
Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
    50                  55                  60

TTA ACA CAG TCT ATG GCC ATC ATA CGT TAT ATA GCT GAC AAG CAC AAC       240
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65                  70                  75                  80

ATG TTG GGT GGT TGT CCA AAA GAG CGT GCA GAG ATT TCA ATG CTT GAA       288
Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
                85                  90                  95

GGA GCG GTT TTG GAT ATT AGA TAC GGT GTT TCG AGA ATT GCA TAT AGT       336
Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
            100                 105                 110

AAA GAC TTT GAA ACT CTC AAA GTT GAT TTT CTT AGC AAG CTA CCT GAA       384
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
        115                 120                 125

ATG CTG AAA ATG TTC GAA GAT CGT TTA TGT CAT AAA ACA TAT TTA AAT       432
Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
    130                 135                 140

GGT GAT CAT GTA ACC CAT CCT GAC TTC ATG TTG TAT GAC GCT CTT GAT       480
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145                 150                 155                 160
```

```
GTT GTT TTA TAC ATG GAC CCA ATG TGC CTG GAT GCG TTC CCA AAA TTA          528
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
                165                 170                 175

GTT TGT TTT AAA AAA CGT ATT GAA GCT ATC CCA CAA ATT GAT AAG TAC          576
Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
            180                 185                 190

TTG AAA TCC AGC AAG TAT ATA GCA TGG CCT TTG CAG GGC TGG CAA GCC          624
Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
        195                 200                 205

ACG TTT GGT GGT GGC GAC CAT CCT CCA AAA TCG GAT CTG GTT CCG CGT          672
Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
    210                 215                 220

GGA TCC CCG TCC CAG CTC CAA CAG GCA GAA AAT AAT ATC ACT AAT TCC          720
Gly Ser Pro Ser Gln Leu Gln Gln Ala Glu Asn Asn Ile Thr Asn Ser
225                 230                 235                 240

AAA AAA GAA ATG ACA AAG CTA CGA GAA AAA GTG AAA AAG GCC GAG AAA          768
Lys Lys Glu Met Thr Lys Leu Arg Glu Lys Val Lys Lys Ala Glu Lys
                245                 250                 255

GAA AAA TTG GAC GCC ATT AAC CGG GCA ACC AAG CTG GAA GAG GAA CGA          816
Glu Lys Leu Asp Ala Ile Asn Arg Ala Thr Lys Leu Glu Glu Glu Arg
            260                 265                 270

AAC CAA GCG TAC AAA GCA GCA CAC AAG GCA GAG GAG GAA AAG GCT AAA          864
Asn Gln Ala Tyr Lys Ala Ala His Lys Ala Glu Glu Glu Lys Ala Lys
        275                 280                 285

ACA TTT CAA CGC CTT ATA ACA TTT GAG TCG GAA AAT ATT AAC TTA AAG          912
Thr Phe Gln Arg Leu Ile Thr Phe Glu Ser Glu Asn Ile Asn Leu Lys
    290                 295                 300

AAA AGG CCA AAT GAC GCA GTT TCA AAT CGG GAT AAG AAA AAA AAT TCT          960
Lys Arg Pro Asn Asp Ala Val Ser Asn Arg Asp Lys Lys Lys Asn Ser
305                 310                 315                 320

GAA ACC GCA AAA ACT GAC GAA GTA GAG AAA CAG AGG GCG GCT GAG GCT         1008
Glu Thr Ala Lys Thr Asp Glu Val Glu Lys Gln Arg Ala Ala Glu Ala
                325                 330                 335

GCC AAG GCC GTG GAG ACG GAG AAG CAG AGG GCA GCT GAG GCC ACG AAG         1056
Ala Lys Ala Val Glu Thr Glu Lys Gln Arg Ala Ala Glu Ala Thr Lys
            340                 345                 350

GTT GCC GAA GCG GAG AAG CGG AAG GCA GCT GAG GCC GCC AAG GCC GTG         1104
Val Ala Glu Ala Glu Lys Arg Lys Ala Ala Glu Ala Ala Lys Ala Val
        355                 360                 365

GAG ACG GAG AAG CAG AGG GCA GCT GAA GCC ACG AAG GTT GCC GAA GCG         1152
Glu Thr Glu Lys Gln Arg Ala Ala Glu Ala Thr Lys Val Ala Glu Ala
    370                 375                 380

GAG AAG CAG AAG GCA GCT GAG GCC GCC AAG GCC GTG GAG ACG GAG AAG         1200
Glu Lys Gln Lys Ala Ala Glu Ala Ala Lys Ala Val Glu Thr Glu Lys
385                 390                 395                 400

CAG AGG GCA GCT GAA GCC ACG AAG GTT GCC GAA GCG GAG AAG CAG AGG         1248
Gln Arg Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Arg
                405                 410                 415

GCA GCT GAA GCC ATG AAG GTT GCC GAA GCG GAG AAG CAG AAG GCA GCT         1296
Ala Ala Glu Ala Met Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala
            420                 425                 430
```

```
GAG GCC ACG AAG GTT GCC GAA GCG GAG AAG CAG AAG GCA GCT GAA GCC      1344
Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala
        435                 440                 445

ACG AAG GTT GCC GAA GCG GAG AAG CAG AAG GCA GCT GAA GCC ACG AAG      1392
Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys
    450                 455                 460

GTT GCC GAA GCG GAG AAG CAG AAG GCA GCT GAA GCC ACG AAG GTT GCC      1440
Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala
465                 470                 475                 480

GAA GCG GAG AAG CAG AAG GCA GCT GAA GCC ACG AAG GTT GCC GAA GCG      1488
Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala Glu Ala
                485                 490                 495

GAG AAG CAG AAG GCA GCT GAA GCC ACG AAG GTT GCC GAA GCG GAG AAG      1536
Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys
            500                 505                 510

CAG AAG GCA GCT GAA GCC ACG AAG GTT GCC GAA GCG GAG AAG CAG AAG      1584
Gln Lys Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys
        515                 520                 525

GCA GCT GAA GCC ACG AAG GTT GCC GAA GCG GAG AAG CAG AAG GCA GCT      1632
Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala
    530                 535                 540

GAA GCC ACG AAG GTT GCC GAA GCG GAG AAG CAG AAG GCA GCT GAA GCT      1680
Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala
545                 550                 555                 560

GCC AAG GCT ATG GAG TCG CAG AAG CAG AGA TTC TTA GAA CGT TTT GCG      1728
Ala Lys Ala Met Glu Ser Gln Lys Gln Arg Phe Leu Glu Arg Phe Ala
                565                 570                 575

GTT CTT GAG GAG GAG AAA AAG GCA GCC TTA AGA GCG GCG GAG ATG GAG      1776
Val Leu Glu Glu Glu Lys Lys Ala Ala Leu Arg Ala Ala Glu Met Glu
            580                 585                 590

AGG AGG AAA ATA ACA AAC ATA ATG AAG AAT AAA GGT GTA CGC AGT TCG      1824
Arg Arg Lys Ile Thr Asn Ile Met Lys Asn Lys Gly Val Arg Ser Ser
        595                 600                 605

GAT TCG GTG CCG CTT GTG GAG GGG AAT CGC TCT GTT ACT GAG AGT TCT      1872
Asp Ser Val Pro Leu Val Glu Gly Asn Arg Ser Val Thr Glu Ser Ser
    610                 615                 620

TGT AGA AAT CGG TTT CGT TTT TGT AGA AAT CGG TTT CGT TTT TCA TGT      1920
Cys Arg Asn Arg Phe Arg Phe Cys Arg Asn Arg Phe Arg Phe Ser Cys
625                 630                 635                 640

TCT GTA ATG TGA                                                     1932
Ser Val Met
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 643 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro
 1               5                  10                  15

Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
            20                  25                  30

Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
        35                  40                  45

Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
    50                  55                  60

Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65                  70                  75                  80

Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
                85                  90                  95

Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
            100                 105                 110

Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
        115                 120                 125

Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
    130                 135                 140

Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145                 150                 155                 160

Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
                165                 170                 175

Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
            180                 185                 190

Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
        195                 200                 205

Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
    210                 215                 220

Gly Ser Pro Ser Gln Leu Gln Gln Ala Glu Asn Asn Ile Thr Asn Ser
225                 230                 235                 240

Lys Lys Glu Met Thr Lys Leu Arg Glu Lys Val Lys Lys Ala Glu Lys
                245                 250                 255

Glu Lys Leu Asp Ala Ile Asn Arg Ala Thr Lys Leu Glu Glu Glu Arg
            260                 265                 270

Asn Gln Ala Tyr Lys Ala Ala His Lys Ala Glu Glu Glu Lys Ala Lys
        275                 280                 285

Thr Phe Gln Arg Leu Ile Thr Phe Glu Ser Glu Asn Ile Asn Leu Lys
    290                 295                 300

Lys Arg Pro Asn Asp Ala Val Ser Asn Arg Asp Lys Lys Lys Asn Ser
305                 310                 315                 320

Glu Thr Ala Lys Thr Asp Glu Val Glu Lys Gln Arg Ala Ala Glu Ala
                325                 330                 335

Ala Lys Ala Val Glu Thr Glu Lys Gln Arg Ala Ala Glu Ala Thr Lys
            340                 345                 350

Val Ala Glu Ala Glu Lys Arg Lys Ala Ala Glu Ala Ala Lys Ala Val
```

```
        355                 360                 365

Glu Thr Glu Lys Gln Arg Ala Ala Glu Ala Thr Lys Val Ala Glu Ala
    370                 375                 380

Glu Lys Gln Lys Ala Ala Glu Ala Ala Lys Ala Val Glu Thr Glu Lys
385                 390                 395                 400

Gln Arg Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Arg
                405                 410                 415

Ala Ala Glu Ala Met Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala
            420                 425                 430

Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala
        435                 440                 445

Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys
    450                 455                 460

Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala
465                 470                 475                 480

Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala Glu Ala
                485                 490                 495

Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys
            500                 505                 510

Gln Lys Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys
        515                 520                 525

Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala
    530                 535                 540

Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala
545                 550                 555                 560

Ala Lys Ala Met Glu Ser Gln Lys Gln Arg Phe Leu Glu Arg Phe Ala
                565                 570                 575

Val Leu Glu Glu Glu Lys Lys Ala Ala Leu Arg Ala Ala Glu Met Glu
            580                 585                 590

Arg Arg Lys Ile Thr Asn Ile Met Lys Asn Lys Gly Val Arg Ser Ser
        595                 600                 605

Asp Ser Val Pro Leu Val Glu Gly Asn Arg Ser Val Thr Glu Ser Ser
    610                 615                 620

Cys Arg Asn Arg Phe Arg Phe Cys Arg Asn Arg Phe Arg Phe Ser Cys
625                 630                 635                 640

Ser Val Met
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1419 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1416

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
ATG TCC CCT ATA CTA GGT TAT TGG AAA ATT AAG GGC CTT GTG CAA CCC      48
Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro
 1               5                  10                  15

ACT CGA CTT CTT TTG GAA TAT CTT GAA GAA AAA TAT GAA GAG CAT TTG      96
Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
            20                  25                  30

TAT GAG CGC GAT GAA GGT GAT AAA TGG CGA AAC AAA AAG TTT GAA TTG     144
Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
        35                  40                  45

GGT TTG GAG TTT CCC AAT CTT CCT TAT TAT ATT GAT GGT GAT GTT AAA     192
Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
    50                  55                  60

TTA ACA CAG TCT ATG GCC ATC ATA CGT TAT ATA GCT GAC AAG CAC AAC     240
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65                  70                  75                  80

ATG TTG GGT GGT TGT CCA AAA GAG CGT GCA GAG ATT TCA ATG CTT GAA     288
Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
                85                  90                  95

GGA GCG GTT TTG GAT ATT AGA TAC GGT GTT TCG AGA ATT GCA TAT AGT     336
Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
            100                 105                 110

AAA GAC TTT GAA ACT CTC AAA GTT GAT TTT CTT AGC AAG CTA CCT GAA     384
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
        115                 120                 125

ATG CTG AAA ATG TTC GAA GAT CGT TTA TGT CAT AAA ACA TAT TTA AAT     432
Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
    130                 135                 140

GGT GAT CAT GTA ACC CAT CCT GAC TTC ATG TTG TAT GAC GCT CTT GAT     480
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145                 150                 155                 160

GTT GTT TTA TAC ATG GAC CCA ATG TGC CTG GAT GCG TTC CCA AAA TTA     528
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
                165                 170                 175

GTT TGT TTT AAA AAA CGT ATT GAA GCT ATC CCA CAA ATT GAT AAG TAC     576
Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
            180                 185                 190

TTG AAA TCC AGC AAG TAT ATA GCA TGG CCT TTG CAG GGC TGG CAA GCC     624
Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
        195                 200                 205

ACG TTT GGT GGT GGC GAC CAT CCT CCA AAA TCG GAT CTG ATC GAA GGT     672
Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Ile Glu Gly
    210                 215                 220

CGT GGG ATC CCC CCG GGC TGC AGG AAT TCC ACG AAG GTT GCC GAA GCG     720
Arg Gly Ile Pro Pro Gly Cys Arg Asn Ser Thr Lys Val Ala Glu Ala
225                 230                 235                 240
```

```
GAG AAG CAG AAG GCA GCT GAA GCC ACG AAG GTT GCC GAA GCG GAG AAG          768
Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys
                245                 250                 255

CAG AGG GCA GCT GAA GCC ACG AAG GTT GCC GAA GCG GAG AAG CAG AAG          816
Gln Arg Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys
            260                 265                 270

GCA GCT GAA GCC ACG AAG GTT GCC GAA GCG GAG AAG CAG AGG GCA GCT          864
Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Arg Ala Ala
        275                 280                 285

GAA GCC ACG AAG GTT GCC GAA GCG GAG AAG CAA AAG GCA GCT GAG GCC          912
Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala
    290                 295                 300

ACG AAG GTT GCC GGA GAC GAG AAG CAG AAG GCA GCT GAA GCC ACG AAG          960
Thr Lys Val Ala Gly Asp Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys
305                 310                 315                 320

GTT GCC GAA GCG GAG AAG CAG AAG GCA GCT GAA GCC ACG AAG GTT GCC         1008
Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala
                325                 330                 335

GAA GCG GAG AAG CAG AAG GCA GCT GAA GCC ACG AAG GTT GCC GAA GCG         1056
Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala Glu Ala
            340                 345                 350

GAG AAG CAG AAG GCA GCT GAA GCC ACG AAG GTT GCC GAA GCG GAG AAG         1104
Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys
        355                 360                 365

CAG AAG GCA GCT GAA GCC ACG AAG GTT GCC GAA GCG GAG AAG CAG AAG         1152
Gln Lys Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys
    370                 375                 380

GCA GCT GAA GCC ACG AAG GTT GCC GAA GCG GAG AAG CAG AAG GCA GCT         1200
Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala
385                 390                 395                 400

GAA GCC ACG AAG GTT GCC GAA GCG GAG AAG CAG AAG GCA GCT GAA GCC         1248
Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala
                405                 410                 415

ACG AAG GTT GCC GAA GCG GAG AAG CAG AAG GCA GCT GAA GCC ACG AAG         1296
Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys
            420                 425                 430

GTT GCC GAA GCG GAG AAG CAG AAG GCA GCT GAA GCC ACG AAG GTT GCC         1344
Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala
        435                 440                 445

GAA GCG GAG AAG CAG AAG GTA GGT GAG GCT GAT CAA GCT TAT CGA TAC         1392
Glu Ala Glu Lys Gln Lys Val Gly Glu Ala Asp Gln Ala Tyr Arg Tyr
    450                 455                 460

CGT CGG GAA TTC ATC GTG ACT GAC TGA                                    1419
Arg Arg Glu Phe Ile Val Thr Asp
465                 470
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 472 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro
 1               5                  10                  15

Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
            20                  25                  30

Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
        35                  40                  45

Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
    50                  55                  60

Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65                  70                  75                  80

Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
                85                  90                  95

Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
            100                 105                 110

Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
        115                 120                 125

Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
    130                 135                 140

Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145                 150                 155                 160

Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
                165                 170                 175

Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
            180                 185                 190

Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
        195                 200                 205

Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Ile Glu Gly
    210                 215                 220

Arg Gly Ile Pro Pro Gly Cys Arg Asn Ser Thr Lys Val Ala Glu Ala
225                 230                 235                 240

Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys
                245                 250                 255

Gln Arg Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys
            260                 265                 270

Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Arg Ala Ala
        275                 280                 285

Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala
    290                 295                 300

Thr Lys Val Ala Gly Asp Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys
305                 310                 315                 320

Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala
                325                 330                 335
```

Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala Glu Ala
340 345 350

Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys
355 360 365

Gln Lys Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys
370 375 380

Ala Ala Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala
385 390 395 400

Glu Ala Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala
405 410 415

Thr Lys Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys
420 425 430

Val Ala Glu Ala Glu Lys Gln Lys Ala Ala Glu Ala Thr Lys Val Ala
435 440 445

Glu Ala Glu Lys Gln Lys Val Gly Glu Ala Asp Gln Ala Tyr Arg Tyr
450 455 460

Arg Arg Glu Phe Ile Val Thr Asp
465 470

WHAT WE CLAIM IS:

1. A polypeptide having a sequence that corresponds to the amino acid sequence of at least one of the C-terminal and N-terminal nonrepetitive regions of the TCR27 protein.

2. A polypeptide as claimed in Claim 1, wherein said polypeptide comprises an amino acid sequence of one or more repeats from the central region of the TCR27 protein.

3. A polypeptide as claimed in Claim 2, wherein said polypeptide corresponds to the N-terminal nonrepetitive region of the TCR27 protein and at least one repeat from the central region of the TCR27 protein, and does not correspond to the C-terminal nonrepetitive region.

4. A polypeptide as claimed in Claim 1, additionally comprising a linker sequence at either the N-terminus or the C-terminus to facilitate attachment or conjugation of said polypeptide to a carrier molecule in a liquid or solid support system.

5. A polypeptide as claimed in Claim 2, additionally comprising a linker sequence at either the N-terminus or the C-terminus to facilitate attachment or conjugation of said polypeptide to a carrier molecule in a liquid or solid support system.

6. A polypeptide as claimed in Claim 3, additionally comprising a linker sequence at either the N-terminus or the C-terminus to facilitate attachment or conjugation of said polypeptide to a carrier molecule in a liquid or solid support system.

7. A polypeptide as claimed in Claim 1, wherein said polypeptide is substantially pure.

8. A polypeptide as claimed in Claim 2, wherein said polypeptide is substantially pure.

9. A polypeptide as claimed in Claim 3, wherein said polypeptide is substantially pure.

10. An isolated polynucleotide encoding a polypeptide as claimed in Claim 1.

11. An isolated polynucleotide encoding a polypeptide as claimed in Claim 2.

12. An isolated polynucleotide encoding a polypeptide as claimed in Claim 3.

13. A cell transformed with a recombinant plasmid that expresses a polypeptide as claimed in Claim 1.

14. A cell transformed with a recombinant plasmid that expresses a polypeptide as claimed in Claim 2.

15. A cell transformed with a recombinant plasmid that expresses a polypeptide as claimed in Claim 3.

16. A method for detecting the presence of antibodies to *T. cruzi* in an individual, comprising the steps of:

contacting a putative anti-*T. cruzi* antibody-containing sample from an individual with a polypeptide as claimed in Claim 1 that is attached or conjugated to a carrier molecule or attached or conjugated to a solid phase;

allowing anti-*T. cruzi* antibodies in said sample to bind to said polypeptide;

washing away unbound anti-*T. cruzi* antibodies; and

adding a compound that enables detection of the anti-*T. cruzi* antibodies which are specifically bound to the polypeptide.

17. A method for detecting the presence of antibodies to *T. cruzi* in an individual, comprising the steps of:

contacting a putative anti-*T. cruzi* antibody-containing sample from an individual with a polypeptide as claimed in Claim 2 that is attached or conjugated to a carrier molecule or attached or conjugated to a solid phase;

allowing anti-*T. cruzi* antibodies in said sample to bind to said polypeptide;

washing away unbound anti-*T. cruzi* antibodies; and

adding a compound that enables detection of the anti-*T. cruzi* antibodies which are specifically bound to the polypeptide.

18. A method for detecting the presence of antibodies to *T. cruzi* in an individual, comprising the steps of:

contacting a putative anti-*T. cruzi* antibody-containing sample from an individual with a polypeptide as claimed in Claim 3 that is attached or conjugated to a carrier molecule or attached or conjugated to a solid phase;

allowing anti-*T. cruzi* antibodies in said sample to bind to said polypeptide;

washing away unbound anti-*T. cruzi* antibodies; and

adding a compound that enables detection of the anti-*T. cruzi* antibodies which are specifically bound to the polypeptide.

19. A method as claimed in Claim 16, wherein the compound that enables detection of the anti-*T. cruzi* antibodies is selected from the group consisting of a colorometric agent, a fluorescent agent, a chemiluminescent agent and a radionuclide.

20. A kit for diagnosing the presence or anti-*T. cruzi* antibodies in a sample, comprising:

a container in which a polypeptide having a sequence that corresponds to the amino acid sequence of at least one of the C-terminal and N-terminal nonrepetitive regions of the TCR27 protein is attached or conjugated to a carrier molecule or attached or conjugated to a solid phase; and

directions for carrying out the method as claimed in Claim 16.

21. A kit as claimed in Claim 18, additionally comprising a container of a compound that binds to anti-*T. cruzi* antibodies and that renders said antibodies detectable.

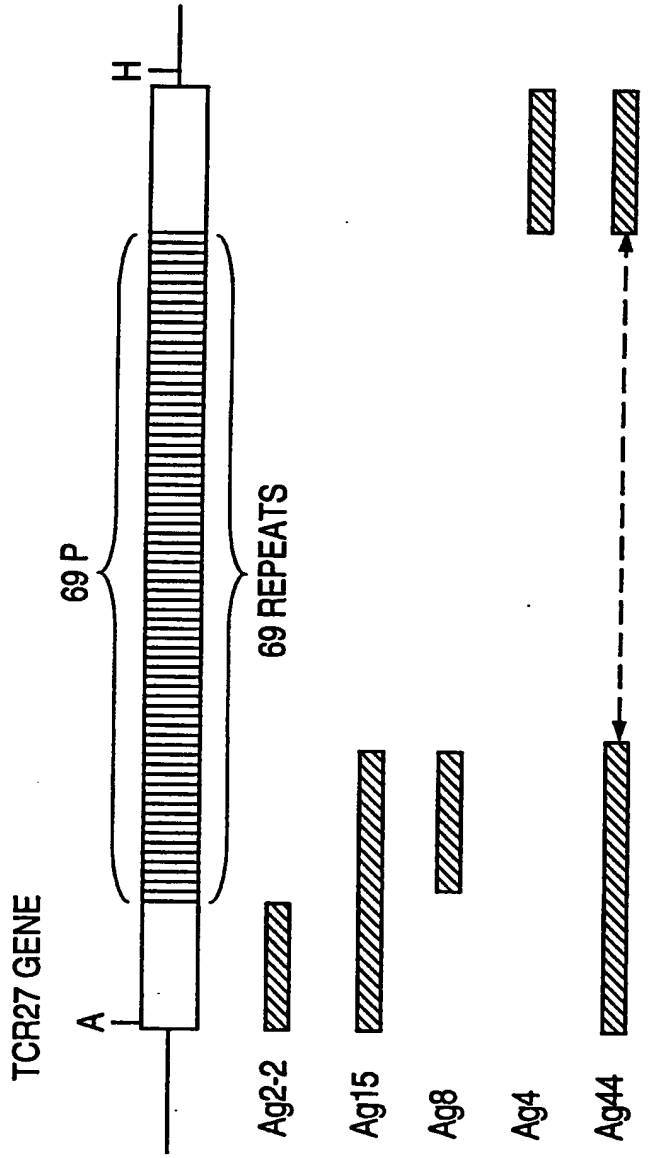


FIG. 1

FIG. 2A-1

```
 M  S  P  I  L  G  Y  W  K  I  K  G  L  V  Q  P  T  R  L  L
ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTT
---------+---------+---------+---------+---------+---------+--------60

 L  E  Y  L  E  E  K  Y  E  E  H  L  Y  E  R  D  E  G  D  K
TTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATAAA
---------+---------+---------+---------+---------+---------+-------120

 W  R  N  K  K  F  E  L  G  L  E  F  P  N  L  P  Y  Y  I  D
TGGCGAAACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATCTTCCTTATTATATTGAT
---------+---------+---------+---------+---------+---------+-------180

 G  D  V  K  L  T  Q  S  M  A  I  I  R  Y  I  A  D  K  H  N
GGTGATGTTAAATTAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAAC
---------+---------+---------+---------+---------+---------+-------240

 M  L  G  G  C  P  K  E  R  A  E  I  S  M  L  E  G  A  V  L
ATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTG
---------+---------+---------+---------+---------+---------+-------300

 D  I  R  Y  G  V  S  R  I  A  Y  S  K  D  F  E  T  L  K  V
GATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACTTTGAAACTCTCAAAGTT
---------+---------+---------+---------+---------+---------+-------360

 D  F  L  S  K  L  P  E  M  L  K  M  F  E  D  R  L  C  H  K
GATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCGAAGATCGTTTATGTCATAAA
---------+---------+---------+---------+---------+---------+-------420

 T  Y  L  N  G  D  H  V  T  H  P  D  F  M  L  Y  D  A  L  D
ACATATTTAAATGGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGAT
---------+---------+---------+---------+---------+---------+-------480

 V  V  L  Y  M  D  P  M  C  L  D  A  F  P  K  L  V  C  F  K
GTTGTTTTATACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAA
---------+---------+---------+---------+---------+---------+-------540

 K  R  I  E  A  I  P  Q  I  D  K  Y  L  K  S  S  K  Y  I  A
AAACGTATTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCA
---------+---------+---------+---------+---------+---------+-------600

 W  P  L  Q  G  W  Q  A  T  F  G  G  G  D  H  P  P  K  S  D
TGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCCAAAATCGGAT
---------+---------+---------+---------+---------+---------+-------660

 L  V  P  R  G  S  P  S  Q  L  Q  Q  A  E  N  N  I  T  N  S
CTGGTTCCGCGTGGATCCCCGTCCCAGCTCCAACAGGCAGAAAATAATATCACTAATTCC
---------+---------+---------+---------+---------+---------+-------720
```

FIG. 2A-2

```
 K  K  E  M  T  K  L  R  E  K  V  K  K  A  E  K  E  K  L  D
AAAAAAGAAATGACAAAGCTACGAGAAAAAGTGAAAAAGGCCGAGAAAGAAAAATTGGAC
---------+---------+---------+---------+---------+-------780

 A  I  N  R  A  T  K  L  E  E  E  R  N  Q  A  Y  K  A  A  H
GCCATTAACCGGGCAACCAAGCTGGAAGAGGAACGAAACCAAGCGTACAAAGCAGCACAC
---------+---------+---------+---------+---------+-------840

 K  A  E  E  E  K  A  K  T  F  Q  R  L  I  T  F  E  S  E  N
AAGGCAGAGGAGGAAAAGGCTAAAACATTTCAACGCCTTATAACATTTGAGTCGGAAAAT
---------+---------+---------+---------+---------+-------900

 I  N  L  K  K  R  P  N  D  A  V  S  N  R  D  K  K  K  N  S
ATTAACTTAAAGAAAAGGCCAAATGACGCAGTTTCAAATCGGGATAAGAAAAAAAATTCT
---------+---------+---------+---------+---------+-------960

E  T  A  K  T  D  E  V  E  K  Q  R  A  A  E  A  A  K  A  V
GAAACCGCAAAAACTGACGAAGTAGAGAAACAGAGGGCGGCTGAGGCTGCCAAGGCCGTG
---------+---------+---------+---------+---------+------1020

 E  T  E  K  Q  R  A  A  E  A  T  K  V  A  E  A  E  K  R  K
GAGACGGAGAAGCAGAGGGCAGCTGAGGCCACGAAGGTTGCCGAAGCGGAGAAGCGGAAG
---------+---------+---------+---------+---------+------1080

 A  A  E  A  A  K  A  V  E  T  E  K  Q  R  A  A  E  A  T  K
GCAGCTGAGGCCGCCAAGGCCGTGGAGACGGAGAAGCAGAGGGCAGCTGAAGCCACGAAG
---------+---------+---------+---------+---------+------1140

 V  A  E  A  E  K  Q  K  A  A  E  A  A  K  A  V  E  T  E  K
GTTGCCGAAGCGGAGAAGCAGAAGGCAGCTGAGGCCGCCAAGGCCGTGGAGACGGAGAAG
---------+---------+---------+---------+---------+------1200

 Q  R  A  A  E  A  T  K  V  A  E  A  E  K  Q  R  A  A  E  A
CAGAGGGCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAGGGCAGCTGAAGCC
---------+---------+---------+---------+---------+------1260

 M  K  V  A  E  A  E  K  Q  K  A  A  E  A  T  K  V  A  E  A
ATGAAGGTTGCCGAAGCGGAGAAGCAGAAGGCAGCTGAGGCCACGAAGGTTGCCGAAGCG
---------+---------+---------+---------+---------+------1320

E  K  Q  K  A  A  E  A  T  K  V  A  E  A  E  K  Q  K  A  A
GAGAAGCAGAAGGCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAAGGCAGCT
---------+---------+---------+---------+---------+------1380

 E  A  T  K  V  A  E  A  E  K  Q  K  A  A  E  A  T  K  V  A
GAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAAGGCAGCTGAAGCCACGAAGGTTGCC
---------+---------+---------+---------+---------+------1440
```

FIG. 2A-3

```
 E  A  E  K  Q  K  A  A  E  A  T  K  V  A  E  A  E  K  Q  K
GAAGCGGAGAAGCAGAAGGCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAAG
---------+---------+---------+---------+---------+------1500

 A  A  E  A  T  K  V  A  E  A  E  K  Q  K  A  A  E  A  T  K
GCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAAGGCAGCTGAAGCCACGAAG
---------+---------+---------+---------+---------+------1560

 V  A  E  A  E  K  Q  K  A  A  E  A  T  K  V  A  E  A  E  K
GTTGCCGAAGCGGAGAAGCAGAAGGCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAG
---------+---------+---------+---------+---------+------1620

 Q  K  A  A  E  A  T  K  V  A  E  A  E  K  Q  K  A  G  E  F
CAGAAGGCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAAGGCAGGGGAATTC
---------+---------+---------+---------+---------+------1680

 I  V  T  D  *
ATCGTGACTGACTGA
---------+-1695
```

FIG. 2B-1

```
 M  S  P  I  L  G  Y  W  K  I  K  G  L  V  Q  P  T  R  L  L
ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTT
---------+---------+---------+---------+---------+---------+--------60

 L  E  Y  L  E  E  K  Y  E  E  H  L  Y  E  R  D  E  G  D  K
TTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATAAA
---------+---------+---------+---------+---------+---------+-------120

 W  R  N  K  K  F  E  L  G  L  E  F  P  N  L  P  Y  Y  I  D
TGGCGAAACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATCTTCCTTATTATATTGAT
---------+---------+---------+---------+---------+---------+-------180

 G  D  V  K  L  T  Q  S  M  A  I  I  R  Y  I  A  D  K  H  N
GGTGATGTTAAATTAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAAC
---------+---------+---------+---------+---------+---------+-------240

 M  L  G  G  C  P  K  E  R  A  E  I  S  M  L  E  G  A  V  L
ATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTG
---------+---------+---------+---------+---------+---------+-------300

 D  I  R  Y  G  V  S  R  I  A  Y  S  K  D  F  E  T  L  K  V
GATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACTTTGAAACTCTCAAAGTT
---------+---------+---------+---------+---------+---------+-------360

 D  F  L  S  K  L  P  E  M  L  K  M  F  E  D  R  L  C  H  K
GATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCGAAGATCGTTTATGTCATAAA
---------+---------+---------+---------+---------+---------+-------420

 T  Y  L  N  G  D  H  V  T  H  P  D  F  M  L  Y  D  A  L  D
ACATATTTAAATGGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGAT
---------+---------+---------+---------+---------+---------+-------480

 V  V  L  Y  M  D  P  M  C  L  D  A  F  P  K  L  V  C  F  K
GTTGTTTTATACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAA
---------+---------+---------+---------+---------+---------+-------540

 K  R  I  E  A  I  P  Q  I  D  K  Y  L  K  S  S  K  Y  I  A
AAACGTATTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCA
---------+---------+---------+---------+---------+---------+-------600

 W  P  L  Q  G  W  Q  A  T  F  G  G  G  D  H  P  P  K  S  D
TGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCCAAAATCGGAT
---------+---------+---------+---------+---------+---------+-------660

 L  V  P  R  G  S  P  S  Q  L  Q  Q  A  E  N  N  I  T  N  S
CTGGTTCCGCGTGGATCCCCGTCCCAGCTCCAACAGGCAGAAAATAATATCACTAATTCC
---------+---------+---------+---------+---------+---------+-------720
```

FIG. 2B-2

```
 K  K  E  M  T  K  L  R  E  K  V  K  K  A  E  K  E  K  L  D
AAAAAAGAAATGACAAAGCTACGAGAAAAAGTGAAAAAGGCCGAGAAAGAAAAATTGGAC
---------+---------+---------+---------+---------+-------780

 A  I  N  R  A  T  K  L  E  E  E  R  N  Q  A  Y  K  A  A  H
GCCATTAACCGGGCAACCAAGCTGGAAGAGGAACGAAACCAAGCGTACAAAGCAGCACAC
---------+---------+---------+---------+---------+-------840

 K  A  E  E  E  K  A  K  T  F  Q  R  L  I  T  F  E  S  E  N
AAGGCAGAGGAGGAAAAGGCTAAAACATTTCAACGCCTTATAACATTTGAGTCGGAAAAT
---------+---------+---------+---------+---------+-------900

I  N  L  K  K  R  P  N  D  A  V  S  N  R  D  K  K  K  N  S
ATTAACTTAAAGAAAAGGCCAAATGACGCAGTTTCAAATCGGGATAAGAAAAAAAATTCT
---------+---------+---------+---------+---------+-------960

 E  T  A  K  T  D  E  V  E  K  Q  R  A  A  E  A  A  K  A  V
GAAACCGCAAAAACTGACGAAGTAGAGAAACAGAGGGCGGCTGAGGCTGCCAAGGCCGTG
---------+---------+---------+---------+---------+------1020

 E  T  E  K  Q  R  A  G  E  F  I  V  T  D  *
GAGACGGAGAAGCAGAGGGCAGGGGAATTCATCGTGACTGACTGA
---------+---------+---------+---------+-1065
```

FIG. 2C-1

```
 M  S  P  I  L  G  Y  W  K  I  K  G  L  V  Q  P  T  R  L  L
ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTT
---------+---------+---------+---------+---------+---------+--------60

 L  E  Y  L  E  E  K  Y  E  E  H  L  Y  E  R  D  E  G  D  K
TTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATAAA
---------+---------+---------+---------+---------+---------+-------120

 W  R  N  K  K  F  E  L  G  L  E  F  P  N  L  P  Y  Y  I  D
TGGCGAAACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATCTTCCTTATTATATTGAT
---------+---------+---------+---------+---------+---------+-------180

 G  D  V  K  L  T  Q  S  M  A  I  I  R  Y  I  A  D  K  H  N
GGTGATGTTAAATTAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAAC
---------+---------+---------+---------+---------+---------+-------240

 M  L  G  G  C  P  K  E  R  A  E  I  S  M  L  E  G  A  V  L
ATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTG
---------+---------+---------+---------+---------+---------+-------300

 D  I  R  Y  G  V  S  R  I  A  Y  S  K  D  F  E  T  L  K  V
GATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACTTTGAAACTCTCAAAGTT
---------+---------+---------+---------+---------+---------+-------360

 D  F  L  S  K  L  P  E  M  L  K  M  F  E  D  R  L  C  H  K
GATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCGAAGATCGTTTATGTCATAAA
---------+---------+---------+---------+---------+---------+-------420

 T  Y  L  N  G  D  H  V  T  H  P  D  F  M  L  Y  D  A  L  D
ACATATTTAAATGGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGAT
---------+---------+---------+---------+---------+---------+-------480

 V  V  L  Y  M  D  P  M  C  L  D  A  F  P  K  L  V  C  F  K
GTTGTTTTATACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAA
---------+---------+---------+---------+---------+---------+-------540

 K  R  I  E  A  I  P  Q  I  D  K  Y  L  K  S  S  K  Y  I  A
AAACGTATTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCA
---------+---------+---------+---------+---------+---------+-------600

 W  P  L  Q  G  W  Q  A  T  F  G  G  G  D  H  P  P  K  S  D
TGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCCAAAATCGGAT
---------+---------+---------+---------+---------+---------+-------660

 P  A  E  A  A  K  A  M  E  S  Q  K  Q  R  F  L  E  R  F  A
CCCCCTGAAGCTGCCAAGGCTATGGAGTCGCAGAAGCAGAGATTCTTAGAACGTTTTGCG
---------+---------+---------+---------+---------+---------+-------720
```

FIG. 2C-2

```
 V  L  E  E  E  K  K  A  A  L  R  A  A  E  M  E  R  R  K  I
GTTCTTGAGGAGGAGAAAAAGGCAGCCTTAAGAGCGGCGGAGATGGAGAGGAGGAAAATA
---------+---------+---------+---------+---------+-------780

 T  N  I  M  K  N  K  G  V  R  S  S  D  S  V  P  L  V  E  G
ACAAACATAATGAAGAATAAAGGTGTACGCAGTTCGGATTCGGTGCCGCTTGTGGAGGGG
---------+---------+---------+---------+---------+-------840

 N  R  S  V  T  E  S  S  C  R  N  R  F  R  F  C  R  N  R  F
AATCGCTCTGTTACTGAGAGTTCTTGTAGAAATCGGTTTCGTTTTTGTAGAAATCGGTTT
---------+---------+---------+---------+---------+-------900

 R  F  S  C  S  V  M  *
CGTTTTTCATGTTCTGTAATGTGA
---------+---------+-924
```

FIG. 2D-1

```
 M  S  P  I  L  G  Y  W  K  I  K  G  L  V  Q  P  T  R  L  L
ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTT
---------+---------+---------+---------+---------+---------60

 L  E  Y  L  E  E  K  Y  E  E  H  L  Y  E  R  D  E  G  D  K
TTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATAAA
---------+---------+---------+---------+---------+-------120

 W  R  N  K  K  F  E  L  G  L  E  F  P  N  L  P  Y  Y  I  D
TGGCGAAACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATCTTCCTTATTATATTGAT
---------+---------+---------+---------+---------+-------180

 G  D  V  K  L  T  Q  S  M  A  I  I  R  Y  I  A  D  K  H  N
GGTGATGTTAAATTAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAAC
---------+---------+---------+---------+---------+-------240

 M  L  G  G  C  P  K  E  R  A  E  I  S  M  L  E  G  A  V  L
ATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTG
---------+---------+---------+---------+---------+-------300

 D  I  R  Y  G  V  S  R  I  A  Y  S  K  D  F  E  T  L  K  V
GATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACTTTGAAACTCTCAAAGTT
---------+---------+---------+---------+---------+-------360

 D  F  L  S  K  L  P  E  M  L  K  M  F  E  D  R  L  C  H  K
GATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCGAAGATCGTTTATGTCATAAA
---------+---------+---------+---------+---------+-------420

 T  Y  L  N  G  D  H  V  T  H  P  D  F  M  L  Y  D  A  L  D
ACATATTTAAATGGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGAT
---------+---------+---------+---------+---------+-------480

 V  V  L  Y  M  D  P  M  C  L  D  A  F  P  K  L  V  C  F  K
GTTGTTTTATACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAA
---------+---------+---------+---------+---------+-------540

 K  R  I  E  A  I  P  Q  I  D  K  Y  L  K  S  S  K  Y  I  A
AAACGTATTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCA
---------+---------+---------+---------+---------+-------600

 W  P  L  Q  G  W  Q  A  T  F  G  G  G  D  H  P  P  K  S  D
TGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCCAAAATCGGAT
---------+---------+---------+---------+---------+-------660

 L  V  P  R  G  S  P  S  Q  L  Q  Q  A  E  N  N  I  T  N  S
CTGGTTCCGCGTGGATCCCCGTCCCAGCTCCAACAGGCAGAAAATAATATCACTAATTCC
---------+---------+---------+---------+---------+-------720
```

FIG. 2D-2

```
 K  K  E  M  T  K  L  R  E  K  V  K  K  A  E  K  E  K  L  D
AAAAAAGAAATGACAAAGCTACGAGAAAAAGTGAAAAAGGCCGAGAAAGAAAAATTGGAC
---------+---------+---------+---------+---------+-------780

 A  I  N  R  A  T  K  L  E  E  E  R  N  Q  A  Y  K  A  A  H
GCCATTAACCGGGCAACCAAGCTGGAAGAGGAACGAAACCAAGCGTACAAAGCAGCACAC
---------+---------+---------+---------+---------+-------840

 K  A  E  E  E  K  A  K  T  F  Q  R  L  I  T  F  E  S  E  N
AAGGCAGAGGAGGAAAAGGCTAAAACATTTCAACGCCTTATAACATTTGAGTCGGAAAAT
---------+---------+---------+---------+---------+-------900

 I  N  L  K  K  R  P  N  D  A  V  S  N  R  D  K  K  K  N  S
ATTAACTTAAAGAAAAGGCCAAATGACGCAGTTTCAAATCGGGATAAGAAAAAAAATTCT
---------+---------+---------+---------+---------+-------960

 E  T  A  K  T  D  E  V  E  K  Q  R  A  A  E  A  A  K  A  V
GAAACCGCAAAAACTGACGAAGTAGAGAAACAGAGGGCGGCTGAGGCTGCCAAGGCCGTG
---------+---------+---------+---------+---------+------1020

 E  T  E  K  Q  R  A  A  E  A  T  K  V  A  E  A  E  K  R  K
GAGACGGAGAAGCAGAGGGCAGCTGAGGCCACGAAGGTTGCCGAAGCGGAGAAGCGGAAG
---------+---------+---------+---------+---------+------1080

 A  A  E  A  A  K  A  V  E  T  E  K  Q  R  A  A  E  A  T  K
GCAGCTGAGGCCGCCAAGGCCGTGGAGACGGAGAAGCAGAGGGCAGCTGAAGCCACGAAG
---------+---------+---------+---------+---------+------1140

 V  A  E  A  E  K  Q  K  A  A  E  A  A  K  A  V  E  T  E  K
GTTGCCGAAGCGGAGAAGCAGAAGGCAGCTGAGGCCGCCAAGGCCGTGGAGACGGAGAAG
---------+---------+---------+---------+---------+------1200

 Q  R  A  A  E  A  T  K  V  A  E  A  E  K  Q  R  A  A  E  A
CAGAGGGCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAGGGCAGCTGAAGCC
---------+---------+---------+---------+---------+------1260

 M  K  V  A  E  A  E  K  Q  K  A  A  E  A  T  K  V  A  E  A
ATGAAGGTTGCCGAAGCGGAGAAGCAGAAGGCAGCTGAGGCCACGAAGGTTGCCGAAGCG
---------+---------+---------+---------+---------+------1320

 E  K  Q  K  A  A  E  A  T  K  V  A  E  A  E  K  Q  K  A  A
GAGAAGCAGAAGGCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAAGGCAGCT
---------+---------+---------+---------+---------+------1380

 E  A  T  K  V  A  E  A  E  K  Q  K  A  A  E  A  T  K  V  A
GAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAAGGCAGCTGAAGCCACGAAGGTTGCC
---------+---------+---------+---------+---------+------1440
```

FIG. 2D-3

```
 E  A  E  K  Q  K  A  A  E  A  T  K  V  A  E  A  E  K  Q  K
GAAGCGGAGAAGCAGAAGGCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAAG
---------+---------+---------+---------+---------+------1500

 A  A  E  A  T  K  V  A  E  A  E  K  Q  K  A  A  E  A  T  K
GCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAAGGCAGCTGAAGCCACGAAG
---------+---------+---------+---------+---------+------1560

 V  A  E  A  E  K  Q  K  A  A  E  A  T  K  V  A  E  A  E  K
GTTGCCGAAGCGGAGAAGCAGAAGGCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAG
---------+---------+---------+---------+---------+------1620

 Q  K  A  A  E  A  T  K  V  A  E  A  E  K  Q  K  A  A  E  A
CAGAAGGCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAAGGCAGCTGAAGCT
---------+---------+---------+---------+---------+------1680

 A  K  A  M  E  S  Q  K  Q  R  F  L  E  R  F  A  V  L  E  E
GCCAAGGCTATGGAGTCGCAGAAGCAGAGATTCTTAGAACGTTTTGCGGTTCTTGAGGAG
---------+---------+---------+---------+---------+------1740

 E  K  K  A  A  L  R  A  A  E  M  E  R  R  K  I  T  N  I  M
GAGAAAAAGGCAGCCTTAAGAGCGGCGGAGATGGAGAGGAGGAAAATAACAAACATAATG
---------+---------+---------+---------+---------+------1800

 K  N  K  G  V  R  S  S  D  S  V  P  L  V  E  G  N  R  S  V
AAGAATAAAGGTGTACGCAGTTCGGATTCGGTGCCGCTTGTGGAGGGGAATCGCTCTGTT
---------+---------+---------+---------+---------+------1860

 T  E  S  S  C  R  N  R  F  R  F  C  R  N  R  F  R  F  S  C
ACTGAGAGTTCTTGTAGAAATCGGTTTCGTTTTTGTAGAAATCGGTTTCGTTTTTCATGT
---------+---------+---------+---------+---------+------1920

 S  V  M  *
TCTGTAATGTGA
--------1932
```

FIG. 2E-1

```
 M  S  P  I  L  G  Y  W  K  I  K  G  L  V  Q  P  T  R  L  L
ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTT
---------+---------+---------+---------+---------+---------+--------60

 L  E  Y  L  E  E  K  Y  E  E  H  L  Y  E  R  D  E  G  D  K
TTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATAAA
---------+---------+---------+---------+---------+---------+-------120

 W  R  N  K  K  F  E  L  G  L  E  F  P  N  L  P  Y  Y  I  D
TGGCGAAACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATCTTCCTTATTATATTGAT
---------+---------+---------+---------+---------+---------+-------180

 G  D  V  K  L  T  Q  S  M  A  I  I  R  Y  I  A  D  K  H  N
GGTGATGTTAAATTAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAAC
---------+---------+---------+---------+---------+---------+-------240

 M  L  G  G  C  P  K  E  R  A  E  I  S  M  L  E  G  A  V  L
ATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTG
---------+---------+---------+---------+---------+---------+-------300

 D  I  R  Y  G  V  S  R  I  A  Y  S  K  D  F  E  T  L  K  V
GATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACTTTGAAACTCTCAAAGTT
---------+---------+---------+---------+---------+---------+-------360

 D  F  L  S  K  L  P  E  M  L  K  M  F  E  D  R  L  C  H  K
GATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCGAAGATCGTTTATGTCATAAA
---------+---------+---------+---------+---------+---------+-------420

 T  Y  L  N  G  D  H  V  T  H  P  D  F  M  L  Y  D  A  L  D
ACATATTTAAATGGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGAT
---------+---------+---------+---------+---------+---------+-------480

 V  V  L  Y  M  D  P  M  C  L  D  A  F  P  K  L  V  C  F  K
GTTGTTTTATACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAA
---------+---------+---------+---------+---------+---------+-------540

 K  R  I  E  A  I  P  Q  I  D  K  Y  L  K  S  S  K  Y  I  A
AAACGTATTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCA
---------+---------+---------+---------+---------+---------+-------600

 W  P  L  Q  G  W  Q  A  T  F  G  G  G  D  H  P  P  K  S  D
TGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCCAAAATCGGAT
---------+---------+---------+---------+---------+---------+-------660

 L  I  E  G  R  G  I  P  P  G  C  R  N  S  T  K  V  A  E  A
CTGATCGAAGGTCGTGGGATCCCCCCGGGCTGCAGGAATTCCACGAAGGTTGCCGAAGCG
---------+---------+---------+---------+---------+---------+-------720
```

FIG. 2E-2

```
 E  K  Q  K  A  A  E  A  T  K  V  A  E  A  E  K  Q  R  A  A
GAGAAGCAGAAGGCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAGGGCAGCT
---------+---------+---------+---------+---------+-------780

 E  A  T  K  V  A  E  A  E  K  Q  K  A  A  E  A  T  K  V  A
GAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAAGGCAGCTGAAGCCACGAAGGTTGCC
---------+---------+---------+---------+---------+-------840

 E  A  E  K  Q  R  A  A  E  A  T  K  V  A  E  A  E  K  Q  K
GAAGCGGAGAAGCAGAGGGCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAAAAG
---------+---------+---------+---------+---------+-------900

 A  A  E  A  T  K  V  A  G  D  E  K  Q  K  A  A  E  A  T  K
GCAGCTGAGGCCACGAAGGTTGCCGGAGACGAGAAGCAGAAGGCAGCTGAAGCCACGAAG
---------+---------+---------+---------+---------+-------960

 V  A  E  A  E  K  Q  K  A  A  E  A  T  K  V  A  E  A  E  K
GTTGCCGAAGCGGAGAAGCAGAAGGCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAG
---------+---------+---------+---------+---------+------1020

 Q  K  A  A  E  A  T  K  V  A  E  A  E  K  Q  K  A  A  E  A
CAGAAGGCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAAGGCAGCTGAAGCC
---------+---------+---------+---------+---------+------1080

 T  K  V  A  E  A  E  K  Q  K  A  A  E  A  T  K  V  A  E  A
ACGAAGGTTGCCGAAGCGGAGAAGCAGAAGGCAGCTGAAGCCACGAAGGTTGCCGAAGCG
---------+---------+---------+---------+---------+------1140

 E  K  Q  K  A  A  E  A  T  K  V  A  E  A  E  K  Q  K  A  A
GAGAAGCAGAAGGCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAAGGCAGCT
---------+---------+---------+---------+---------+------1200

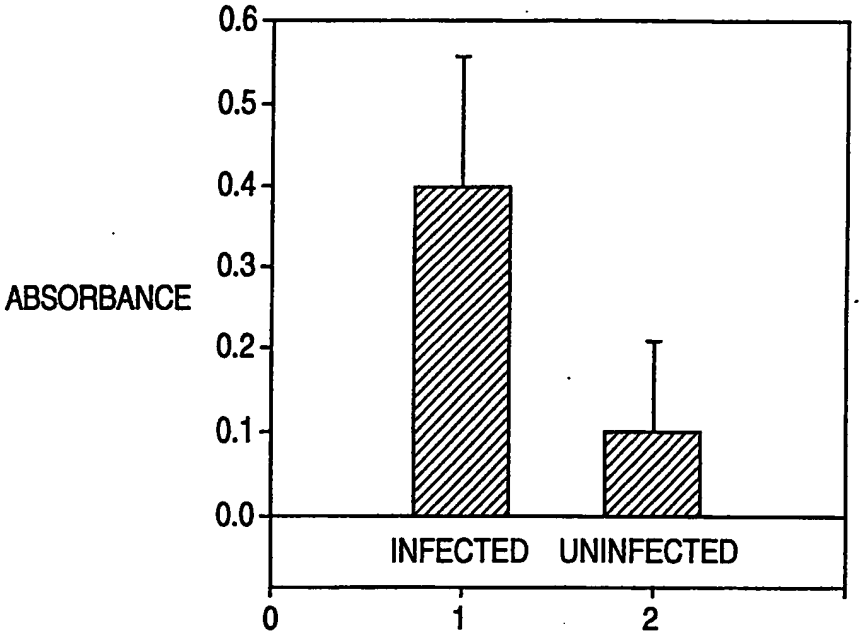
 E  A  T  K  V  A  E  A  E  K  Q  K  A  A  E  A  T  K  V  A
GAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAAGGCAGCTGAAGCCACGAAGGTTGCC
---------+---------+---------+---------+---------+------1260

 E  A  E  K  Q  K  A  A  E  A  T  K  V  A  E  A  E  K  Q  K
GAAGCGGAGAAGCAGAAGGCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAAG
---------+---------+---------+---------+---------+------1320

 A  A  E  A  T  K  V  A  E  A  E  K  Q  K  V  G  E  A  D  Q
GCAGCTGAAGCCACGAAGGTTGCCGAAGCGGAGAAGCAGAAGGTAGGTGAGGCTGATCAA
---------+---------+---------+---------+---------+------1380

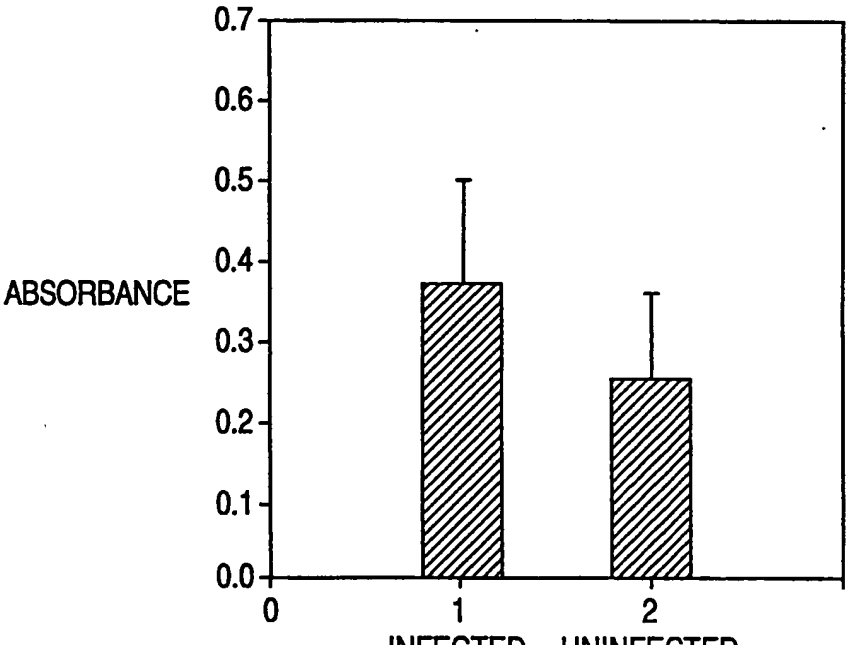
 A  Y  R  Y  R  R  E  F  I  V  T  D  *
GCTTATCGATACCGTCGGGAATTCATCGTGACTGACTGA
---------+---------+---------+-----1419
```

FIG. 3A



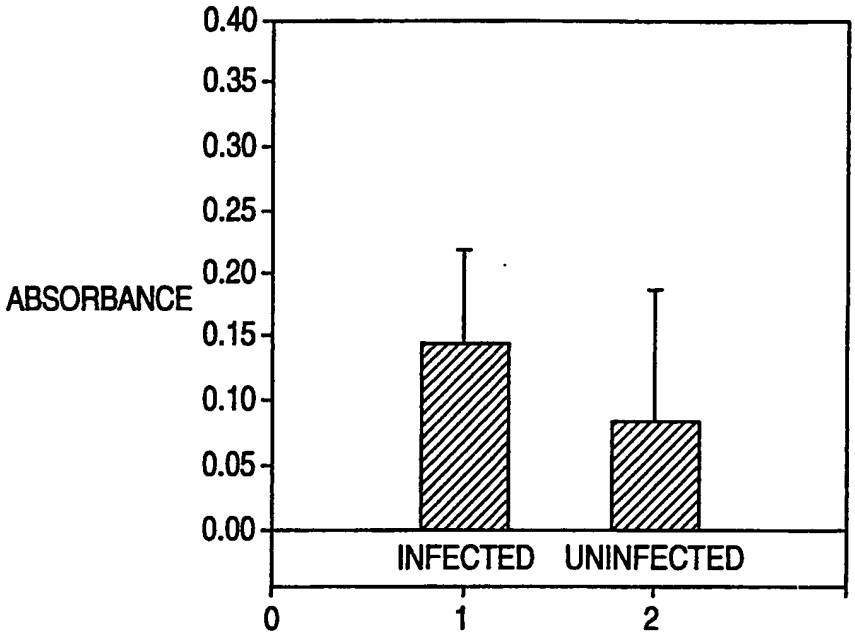
T. CRUZI-INFECTED PATIENTS AND UNINFECTED CONTROLS VS. Ag15

FIG. 3B



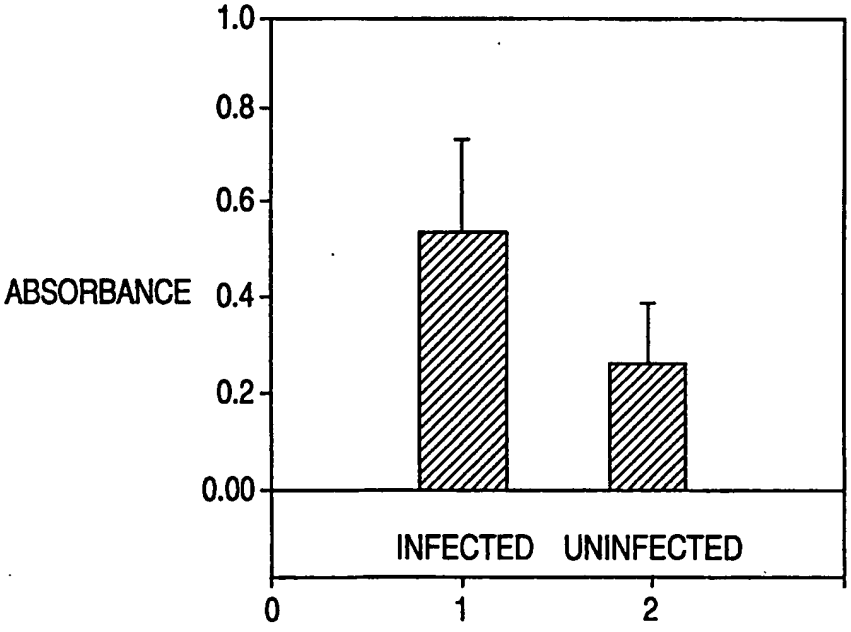
T. CRUZI-INFECTED PATIENTS AND UNINFECTED CONTROLS VS. Ag2-2

FIG. 3C



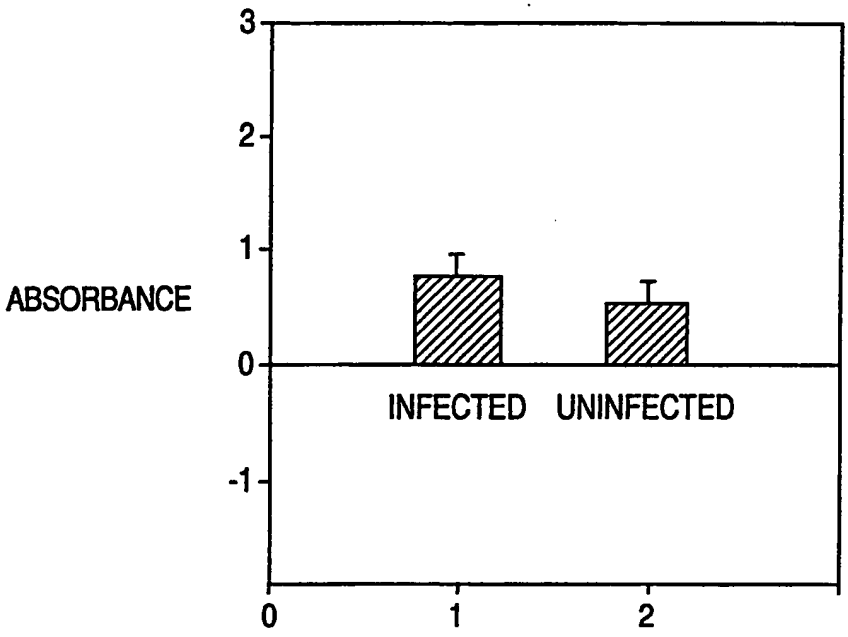
T. CRUZI-INFECTED PATIENTS AND UNINFECTED CONTROLS VS. Ag4

FIG. 3D



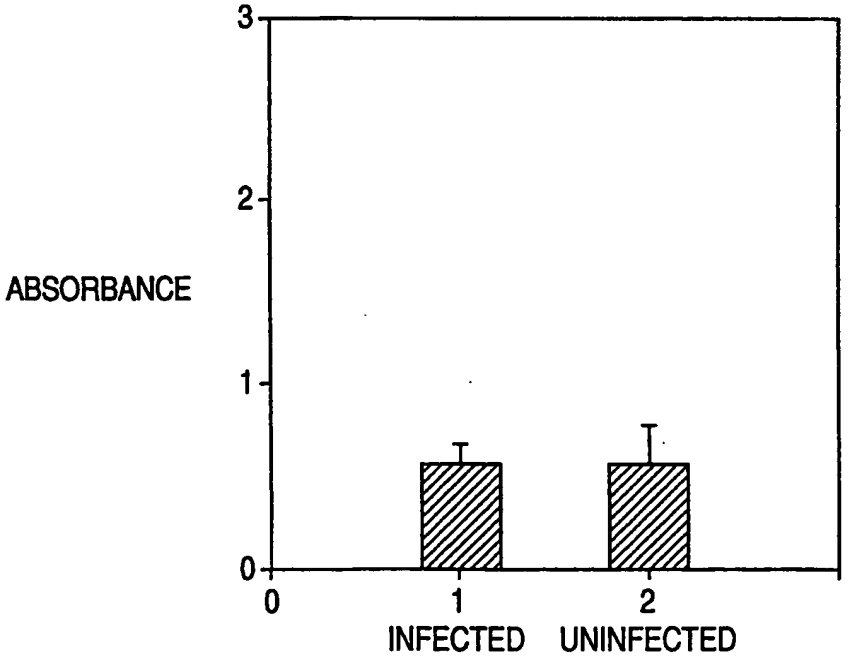
T. CRUZI-INFECTED PATIENTS AND UNINFECTED CONTROLS VS. Ag44

FIG. 3E



T. CRUZI-INFECTED PATIENTS AND UNINFECTED CONTROLS VS. Ag8

FIG. 3F



T. CRUZI-INFECTED PATIENTS AND UNINFECTED CONTROLS VS. GST

INTERNATIONAL SEARCH REPORT

Internatic Application No
PCT/US 95/03191

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/30 C12N15/62 C12N15/54 C12N1/21 C12N9/10
C07K14/44 G01N33/577

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MOL. BIOCHEM. PARASITOL. (1993), 57(2), 317-30 CODEN: MBIPDP;ISSN: 0166-6851, 1993 OTSU, KEIKO ET AL 'Interruption of a Trypanosoma cruzi gene encoding a protein containing 14-amino acid repeats by targeted insertion of the neomycin phosphotransferase gene' cited in the application the whole document --- -/--	1,2,7,8, 10,11, 13,14

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
15 June 1995	29. 06. 95

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Internatic Application No
PCT/US 95/03191

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